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SELECTION FOR BRISTLE NUMBERS IN SOME UNRELATED STRAINS OF DROSOPHILA MELANOGASTER

BY

MARIANNE RASMUSON

(Institute of Genetics, University of Stockholm, Stockholm, Sweden)

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Selection experiments with *Drosophila melanogaster* involving quantitative characters have been undertaken by many investigators. Early reports are those published by MAC DOWELL in 1917, STURTEVANT in 1918 and PAYNE in 1918 and 1920, which all concerned bristle numbers. Selection for facet number in the Bar eye was undertaken by ZELENY (1922). A survey of the earlier investigations is given by MATHER (1941). Lately, penetrating analyses of selection phenomena have been published by MATHER & HARRISON (1949) using bristle numbers and ROBERTSON & REEVE (1952, 1953) using wing and thorax length as selection criteria.

As selection is an irreplaceable method in animal and plant breeding it certainly is of great importance to furnish as broad as possible a basis of experiences and observations concerning it. Furthermore the aims of laboratory selection experiments are numerous and variable. The present investigation primarily concerns the consistency and inconsistency between responses to selection from unrelated strains. As decisive characters two types of bristles have been used. The mean, the variation and the range of phenotypical expression have been

recorded, and also the influences of selection on other characters, as such correlated changes may reveal physiological connections that cannot otherwise be stated.

Another problem that has been investigated is the role of crossing over in selection response. Temperature treatment has been used to exaggerate the crossing over frequency.

Finally the number of gene differences between selection lines has been discussed in connection with crosses between selection lines and continued selection from the crossbred offspring.

MATERIAL AND METHODS

This investigation was started as early as October 1946 with the wild type strain *Örebro*. (About the origin of this and other strains see RASMUSON, 1952.) When subjected to selection the strain had been kept in the laboratory for about 3 generations and mass cultured. The decisive character used was the number of sternopleural bristles. At the start of the experiment the sternopleurals were counted on 25 males and 25 females. The 3 individuals of each sex with the most extreme numbers in both directions were mated together, high with high and low with low, to make the parents of the two selection lines Ör SH (with high bristle number) and Ör SL (with low number). They were cultured in bottles on corn meal medium and kept at 25° C. In the following generation bristles were counted on 25 males and 25 females from each line and selection was continued upwards and downwards with the 2×2 (2 males and 2 females) most extreme individuals as parents. The 2×2 next to the extreme individuals were used as parents for an extra culture in case the first culture did not leave any, or not enough, offspring for selection to be practiced in the next generation. In the latter case the number of animals counted was made complete from the extra culture. The parents were allowed to stay in the bottles until just before the emergence of the next generation. Bristle counts were made when the cultures were 14 days old, that is to say when the oldest flies were 3—4 days old. By this method there was some risk that the females chosen as parents were not virgin. When two or more females had the same extreme bristle number those which appeared youngest were always selected. (This method was most convenient, but may have decreased the intensity of selection, as some of the offspring would not have the selected males as fathers.) From generation 30 of Ör SH and generation 32 of Ör SL the bristle counts were reduced to 20 flies of each sex.

Three other wild type strains, captured shortly before the start of selection, were also selected for high and low number of sternopleurals: *Tunnelgatan, Stäket* and *Algeria* (see RASMUSON 1952), and also the wild type strains

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TABLE 1. Initial conditions and system of notation for the selection lines.

Original strain	Selection character	Direction of selection	Name of selection line	Date for onset of selection	Number of animals counted at the onset of selection
Örebro	Sternopl. br.	low	Ör SL	29.12 1946	2 × 25*
captured	„	high	Ör SH	„	„
in Aug. 1946	Abdominal br.	low	Ör AL	24.11 1949	2(2 × 20)**
	„	high	Ör AH	„	„
	„	low	Ör AL II	2.2 1951	5(2 × 20)
Tunnelgatan	Sternopl. br.	low	Tg SL	9.4 1948	2(2 × 20)
captured	„	high	Tg SH	„	„
in April	Abdominal br.	low	Tg AL	24.11 1949	„
1948	„	high	Tg AH	„	„
Stäket	Sternopl. br.	low	St SL	6.10 1948	3(2 × 20)
captured	„	high	St SH	„	„
in Oct. 1948	Abdominal br.	low	St AL	24.11 1949	2(2 × 20)
	„	high	St AH	„	„
	„	high	St AH II	11.3 1950	„
Algeria	Sternopl. br.	low	Alg SL	16.3 1950	5(2 × 20)
captured	„	high	Alg SH	„	„
in Aug. 1949	Abdominal br.	low	Alg AL	„	„
	„	high	Alg AH	„	„
Florida	Sternopl. br.	low	Fl SL	10.5 1948	2(2 × 20)
introduced	„	high	Fl SH	„	„
around 1920					
Oregon	Sternopl. br.	low	Or SL	9.4 1948	„
introduced	„	high	Or SH	„	„
around 1920					

* 25 males and 25 females.
** 20 males and 20 females from each of 2 cultures.

Florida and *Oregon*, which had been cultured in the laboratory since 1920. The number of flies counted in the original populations at the onset of selection has varied, as seen from table 1, where the initial conditions are stated. The method of selection was the same as for Ör SH and Ör SL, 2 × 20 flies were examined and 2 × 2 were selected.

Besides sternopleurals, another type of bristle has also been used as the criterion of selection, viz. those on the abdominal sternites, used in many selection experiments by MATHER and his collaborators. As in these experiments, the joint number of bristles on the 4th and 5th abdominal segments has been counted. This kind of selection has been practised on the strains Örebro, Tunnelgatan, Stäket and Algeria and was started later than the sternopleural selections (table 1).

RESULTS

I. THE PHENOTYPIC EXPRESSION OF THE SELECTED CHARACTERS

A. Means.

The lines of selection have been followed during varying numbers of generations from 8 to more than 100. The generation means of the selected characters are shown on fig. 1—2. Every point on the diagrams is the mean of male and female means for that generation and is thus usually based on 40 counts.

All the newly captured strains responded to selection by a separation of the high and the low line, but as well the final levels as the number of generations needed to attain maximum effect and stabilisation differ considerably among the lines (table 2).

Örebro. This strain shows the greatest separation between high and low lines of sternopleural selection (fig. 1 a). Ör SL reaches after 8 generations a mean of 11.5 bristles and maintains this with little change until gen. 98,

TABLE 2. Direct results of selection recorded as percentual changes in the mean number of the selected bristle type.

Selection line	Number of selected generations	Final response in % of initial bristle number	Maximum response in % of initial bristle number	Maximum percentual change
Ör SL	105	65	64	36
Tg SL	76	98	85	15
St SL	61	80	79	21
Alg SL	34	86	79	21
Fl SL	12*	92	86	14
Or SL	8*	104	95	5
Ör SH	87**	186	211	111
Tg SH	70	148	159	59
St SH	53	158	166	66
Alg SH	27	119	126	26
Fl SH	14	106	108	8
Or SH	13	110	110	10
Ör AL	21*	41	41	59
Ör AL II	15	89	83	17
Tg AL	41	75	75	25
St AL	35	77	62	38
Alg AL	20	72	67	33
Ör AH	39	106	115	15
Tg AH	41	133	136	36
St AH	16	100	106	6
St AH II	26	114	119	19
Alg AH	34	122	122	22

* Line extinct.
** Sum of original selection and renewed selections from mass mated line.

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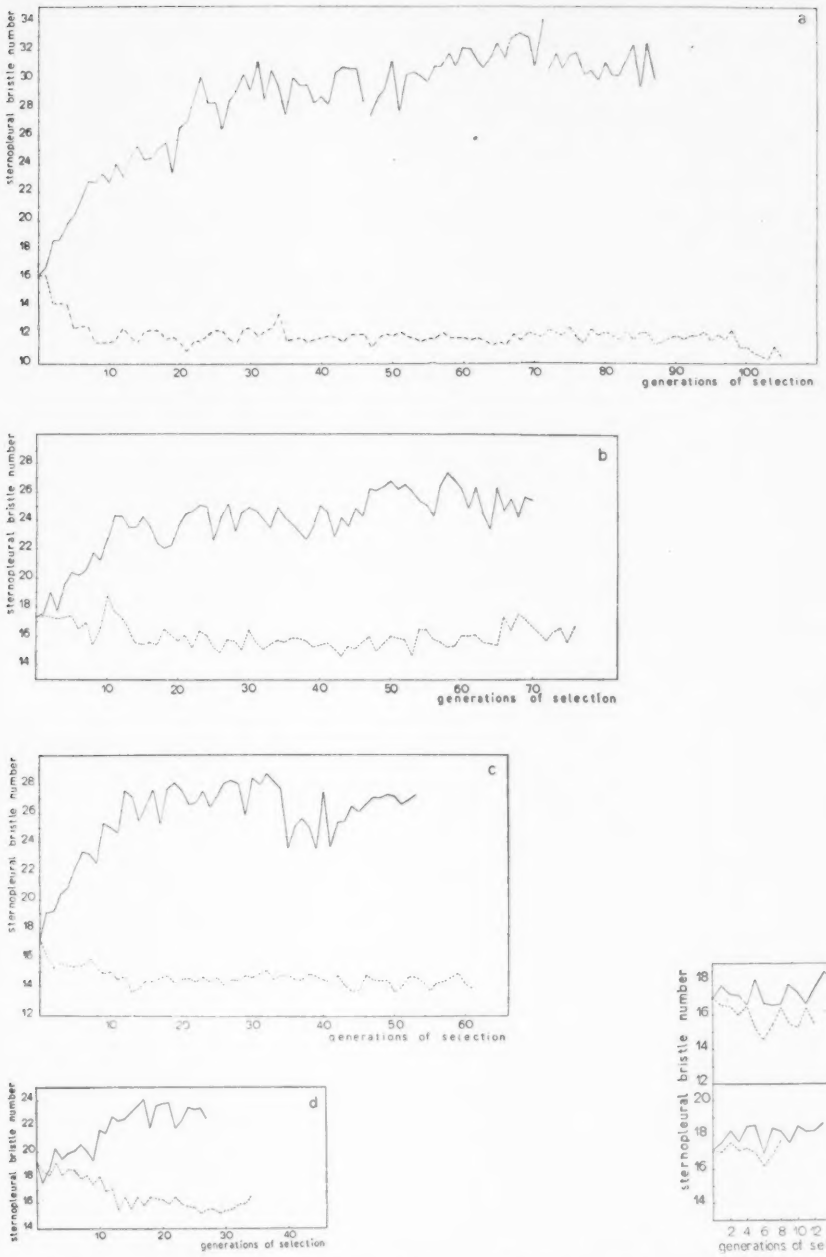

Fig. 1. Responses to sternopleural bristle selection. — Selection for high numbers. - - - - Selection for low numbers. Each point is the mean of male and female mean bristle number. a) Strain Örebro. b) Strain Tunnelgatan. c) Strain Stäket. d) Strain Algeria. e) Laboratory strain Florida. f) Laboratory strain Oregon.

except for a passing increase to 13.5 in gen. 34. From gen. 99 to the end there is, however, an obvious decrease of about 1 bristle, which suggests a real change in the genotype of the line. This decrease is accompanied by a deterioration of fertility.

In the line Ör SH the increase continues for more than 20 generations rather steadily. In gen. 23 the mean was 30.1, which means an increase over the original population of about 85 %. Then the line remained constant, but the fertility was low and in gen. 46 it became extinct. However, from gen. 27 a non-selected mass cultured line had been isolated, and was maintained without bristle counts. When the selected line was lost, selection was started anew from this mass cultured line, which during that time had not appreciably changed its bristle number. In the new line the number of bristles increased slowly for 25 generations, after which time this line also became extinct. It had by then reached a mean 100 % higher than the original level. Selection was renewed once more from the mass cultured line, but without further effect.

Selection for abdominal bristles from the Örebro strain showed quite another trend (fig. 2a). This selection was started nearly 3 years later than the selection for sternopleurals, which means that the strain had been cultured under laboratory conditions for at least another 50 generations. It can be presumed that the potential variability had decreased during this time of relatively constant conditions, but in spite of this there was considerable separation of the lines. The line Ör AL, selected for low number of abdominal bristles, decreased in 11 generations from 42.5 to 22.0 and when the line became extinct in gen. 21 the mean was as low as 17.6, a decrease of almost 60 %. The fertility of this line showed a marked correlation to the bristle number. Flies with low number of bristles often produced few offspring, and selection therefore had to be abandoned in some generations. In gen. 13 no selection could be practised, which resulted in an increase of the mean with 9 bristles in the following generation. Sufficient offspring were now obtained and selection once more reduced the bristle number. This alternation between selected generations with low bristle number and few individuals and non-selected generations that were more numerous and had higher bristle number is, as we shall see, encountered in other AL-selection lines as well.

When the Ör AL-line was lost another selection line for low number of abdominal bristles, Ör AL II, was started from the unselected strain. This line followed the first one rather closely for the first 6 generations, but after that did not decrease any further. On the contrary it almost reverted to the original level in generations 12 and 14. Fertility was good. It is impossible to tell whether the discrepancy between the two AL-lines was due to genetical drift which stopped the selection result too early in Ör AL II or whether the Örebro strain had lost some of its genetical capacity during the time between the two experiments.

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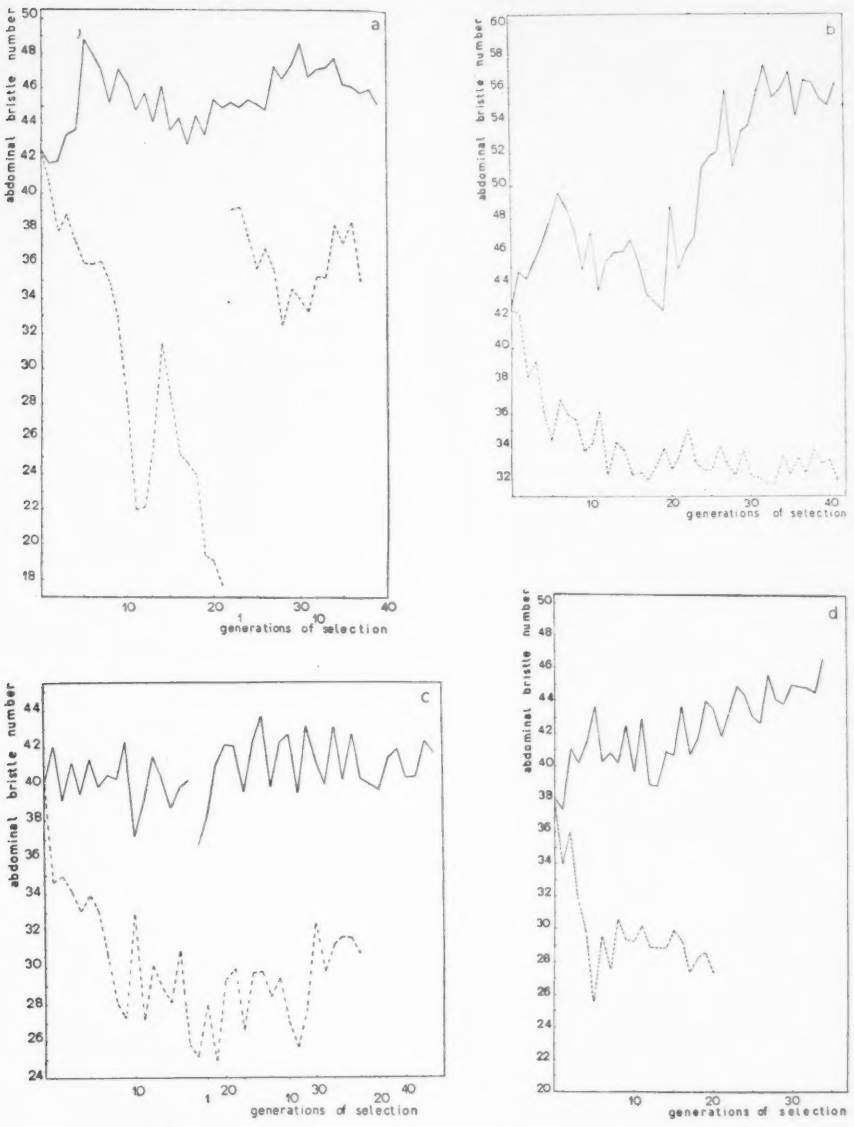

Fig. 2. Responses to abdominal bristle selection. — Selection for high numbers. - - - - Selection for low numbers. Each point is the mean of male and female mean bristle number. a) Strain Örebro. b) Strain Tunnelgatan. c) Strain Stäket. d) Strain Algeria.

Ör AH, the selection line for high number of abdominal bristles, had already reached its maximum value 48.8 by the 5th generation, but later reverted against the pressure of continued selection so much that in gen. 17 it was insignificantly above the level of the original population. Another period of

increase followed which lasted until gen. 30 where the mean was again over 48. The line was however not yet stabilized and decreased steadily until gen. 39 when selection was abolished. Its mean was then 6 % above the original population.

Tunnelgatan. The response to selection for low number of sternopleurals was very small. Flies with missing legs (1 or 2) were occasionally found in this line, Tg SL, which had also rather poor fertility. In gen. 8 a bristle number of 15.5 was reached, 10 % decrease, and after a reversion during generations 10—12 the mean stabilized round 15—16 bristles until gen. 65 where another reversion set in which in generations 66 and 68 brought the mean back to the original level. The last reversion was accompanied by better fertility, which, however, again was lost when the mean decreased after gen. 70.

The mean of the line selected for high number of sternopleural bristles, Tg SH, stabilized after 11 generations at about 23—25 bristles, but rose further after gen. 46 and reached its maximum value 27.3 in gen. 58. From there on it reverted to values around 25.

Selection for low number of abdominal bristles, Tg AL, resulted in a decrease that was completed after 12 generations when the line stabilized about 20—25 % below the origin. The fertility of this line was good, and did not show any correlation with the bristle numbers of the selected flies as in the Ör AL-line.

The upward abdominal selection line, Tg AH, followed at first the same trend as Ör AH; i.e. a rapid increase during 6 generations, and then a reversion towards the level of the original population. From gen. 20 on, however, another increase set in which finally stabilized the line at a mean level between 55 and 57 bristles. This represents an increase of 30—35 % and is the highest level of abdominal bristles encountered in the present experiments.

Stäket. In the sternopleural selection the low line, St SL, reached its lowest value 13.7 in the 13th generation and then its mean kept constant between 14 and 15. The high line, St SH rose rapidly during 12 generations, then slowly until gen. 32 where it reached its highest value, 28.8 (66 % increase). After that the mean suddenly decreased to 24 in 3 generations, and in the same time fertility deteriorated. Another rise resulted after gen. 47 in a stabilization around 27 bristles, but the fertility remained poor.

Selection for low number of abdominal bristles, St AL, brought about a sharp decrease for 9 generations with a mean of 27.3 as result. Fertility also fell off so that the line had to be propagated without selection in some of the following generations. Every time selection was abolished the mean increased, which can be seen from generations 10, 12, 15, 18, 20 etc. The behaviour is parallel to that of Ör AL. After gen. 30 the line seems to stabilize at a mean level of 30—32 bristles.

The St AH line is the only one where selection for high number of ab-

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dominal bristles is completely without effect on the mean. The first attempt, St AH, was started simultaneously with St AL and was continued for 16 generations. The second attempt was made later and the cultures from the original strain then had a mean about 3.5 bristles lower. The line started from these cultures, St AH II, rose in two generations to the same level as St AH, but did not increase beyond this level during 26 generations.

Algeria. This strain had the highest original level for sternopleurals, 19.14. In spite of this the high selection line, Alg SH, did not rise beyond a mean of 23 bristles. The response was rather slow; the maximum value 24.05 appeared in gen. 17. Alg SL, the low line, proceeded still more slowly towards its minimum value, 15.1, reached in gen. 29.

The low abdominal selection line, Alg AL, showed the same rapid decrease of mean and fertility as Ör AL and St AL. Already in gen. 5 the minimum value 25.6 was reached. Moreover, when the line had to be propagated without selection, the result was similarly an increase in mean (generations 6, 8, 11). But after the 5th generation a reversion towards higher number of bristles seems to have taken place in spite of the continued selection.

Similarly the high line, Alg AH, follows Ör AH and Tg AH rather closely; increasing for 5 generations, gradually reverting towards the unselected level, and finally the onset of another period of increase. This second increase which started in gen. 14 was still continuing in gen. 34. The mean was then 46.4, an increase of 22.5 %.

Laboratory strains. Sternopleural selection from the laboratory strains Florida and Oregon had small but significant effects. In the low Florida line the bristle number decreased by 1—2 bristles during the first 6 generations, then remained unchanged until the end of the experiment in gen. 12. The high selection line did not change until the last 2 generations where its mean rose by about 1 bristle. The Oregon lines are not so widely separated and when the low line became extinct in gen. 8 it had not changed its level. The high line was continued until gen. 13 and showed a small but steady increase.

Table 2 gives a survey of the results of selection. Though not all of the lines responded to selection, there is no case where selection has been unable to bring about a separation between opposing lines of selection. Potential variability thus is present everywhere. The correspondance between the selection results and the potential variability of the original population is, however, not to be taken for granted. Even if the intensity of selection is kept constant, chance assortment and linkage conditions and even accumulating mutations during the course of selection may influence the final result. The differences between the newly captured strains and the laboratory strains Florida and Oregon, which have been cultured in laboratory for several decades, are clear, but within shorter periods there is no correlation between generations of culturing preceeding the selection and response to selection.

The rank order of the strains as to selection response is not the same for high and low selection, nor for abdominal and sternopleural selection. On the other hand, some features are found to be common to selection lines of the same kind but of different origin. One is the fact that selection for low number of sternopleurals seldom brings about percentage changes in mean level as great as selection for high number of sternopleurals, whereas the relation between high and low abdominal selection lines is the reverse.

Another thing to be noticed is the common occurance of low fertility and its correlation with the bristle number in the low abdominal selection lines. The only exception here is Tg AL. Finally it is a common trait among the high abdominal selection lines that they do not stabilize on a certain level but that periods of increase and decrease alternate during may generations. In the next chapters the lines will be analysed more closely for intraculture variation, range and stability, and it will be shown that the consistency between selection lines of the same kind will be still more accentuated. Only the selection lines from the strains Örebro, Tunnelgatan, Stäket and Algeria will be considered here.

B. *Intraculture variation of selection lines.*

To avoid the correlation between mean and variation, transformation of the original values to logarithms ($10\ (^{10}\log\frac{x}{10})$) was undertaken before the intraculture variance was estimated (see RASMUSON 1952). By means of this transformation it was possible to compare variation of different selection lines and types of bristles. Mean squares were computed for males and females separately in each generation, but as they showed no different trends the values were pooled. Fig. 3—4 show the changes in variation during the course of selection. For comparison the intraculture variance of the original populations, computed in the same way, has been given.

Sternopleural selection does not bring about any great changes in variation. Two lines, Alg SL and Ör SH, show a small increase in variation during those generations where selection is most effective, but usually there is a decline in variation to a level a little below that of the original population. In some cases a sudden regressing irregularity in mean is accompanied by a high variation (Alg SH 9, Ör SH 26, Ör SL 34, Tg SL 10—12). The cause may be mutations or crossing over which releases new variation. Continued selection may take advantage of the new variation, and the result can sometimes be seen in a further response to selection following the irregularity of variation.

In Ör SL variation is high during generations 100—105 corresponding to the simultaneous decrease in bristle number. The underlying event here seems to be a change of the genotype which reduces bristle number, but which in spite of its selective advantage cannot secure a footing in the population, possibly because of accompanying poor fertility.

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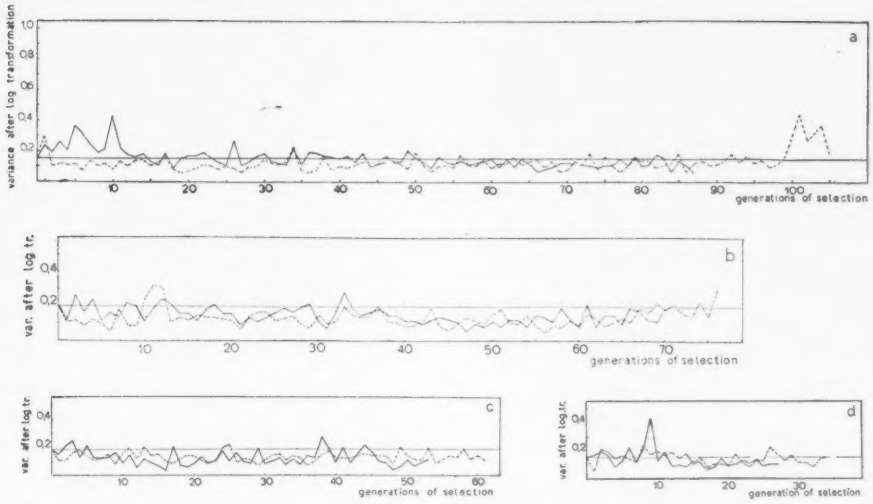


Fig. 3. Selection effects on the variation in sternopleural selection lines. Within generation variances computed after log. transformation of the original values. — High relection lines. - - - how selection lines. For comparision the intraculture variances of the original strains are drawn as horisontal lines.
a) Ör SL and Ör SH. b) Tg SL and Tg SH. c) St SL and St SH. d) Alg SL and Alg SH.

Genotypical changes are thus rather clearly manifested in the variation of sternopleural bristles, but otherwise the variation in these lines is little changed by selection.

Variation in the abdominal selection lines in less consistent. The AH lines show the same picture as the sternopleural selection lines. Release of new variation seems to have occured in Tg AH in generations 11 and 18, in both cases followed by an increase of bristle number.

Of the low lines, Tg AL has the most normal variation. The sharp decrease in mean bristle number during generations 2—5 is accompanied by a high variation, but after that the variance stabilized at the level of the original population, only exceeding it a little between generations 28 and 34.

The other AL selection lines all have abnormally high variation, at least in some generations, and often show the same mirror-image between mean and variance that has been described for the inbred line Örebro b in a previous publication (RASMUSON 1952). This high variation does not in any case appear before generation 5, but is usually manifested when the minimum level of the mean is attained. Nor is it usually maintained during the whole experiment. When St AL stabilized after gen. 30, variation was reduced and similarly it was rather low between generations 7—15 of Alg AL, during which time the mean of this line kept fairly constant. In Ör AL variation decreased in the last two generations before the line became extinct. This decrease was not accompanied by an increase in bristle number; the mean on the contrary being

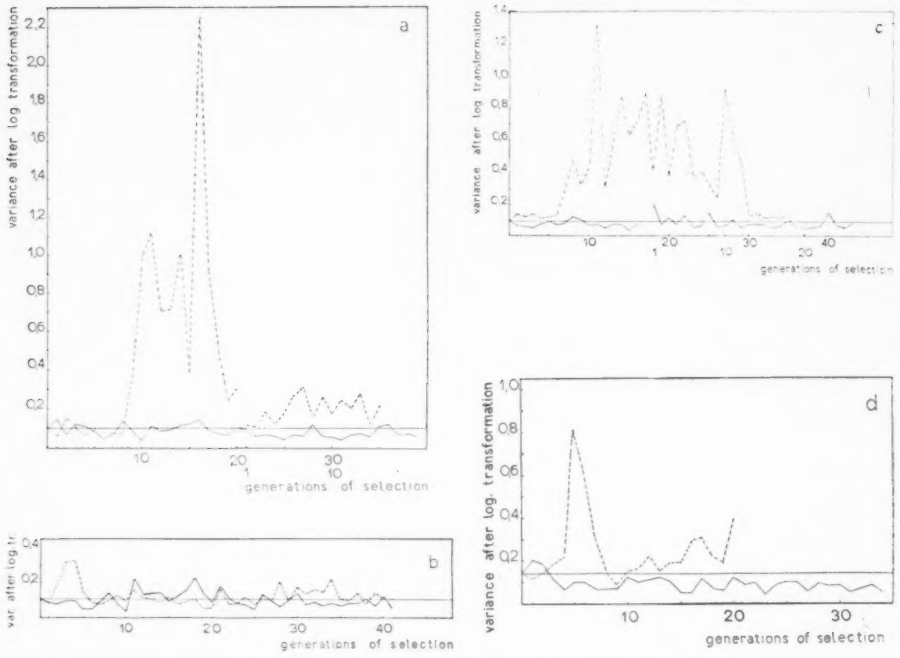


Fig. 4. Selection effects on the variation in abdominal selection lines. Within generation variances, computed after log. transformation of the original values. — High selection lines. - - - Low selection lines. For comparision the intraculture variances of the original strains are drawn as horisontal lines.
a) Ör AL, Ör AL II and Ör AH. b) Tg AL and Tg AH. c) St AL, St AH and St AH II. d) Alg AL and Alg AH.

at its lowest level. Flies with relatively high bristle numbers, which in the previous generations retained both high mean and variance, were not present in these two generations. However, their disappearance was accompanied by a severe infertility, and the line could not be maintained.

The line Ör AL II had a high variance although its mean did not decrease much. The variance did not fluctuate as much as in the three last mentioned lines, nor was any correlation found between mean and variance.

C. *The range.*

Besides the mean and the variation, the range of phenotypical expression may give information about the changes that selection induces. As it is impossible to make use of pooled values from males and females in this case, and no substantial divergencies were found between the sexes, fig. 5 shows the female values only. It will be noted that in the diagrams the original populations are represented by a much greater number of animals (300 for Örebro, 200 for the others) than each generation of the selection lines (usually 20

animals). The chances of finding extreme numbers of bristles are naturally greater when more animals are examined.

The most conspicious thing about the range is that sternopleural selection for low numbers was unable to reduce the bristle number more than one or two bristles below the range of the original population. The low extremes of the SL-lines were mostly represented already in the original population, and selection was effective only in sorting out the high bristle numbers and narrowing the range. There is only one exception, viz. generations 100—105 of Ör SL where the original minimum limit was clearly passed, the extremes going down to 7—8 bristles. As the upper limit remained unchanged the result was a decrease of mean and an increase of range and variation. The minimum limit for the SL-lines was not the same for all the lines. It was lowest for Ör SL, being 9—10 up to generation 99 and thereafter 7—8; highest for Alg SL at 13—14.

In the SH-lines selection was able to change both limits of the range and the high extremes were far beyond those of the original populations. In Ör SH and St SH even the lower extremes passed the high limit of the original population, and no overlapping occurred between these lines and their origins.

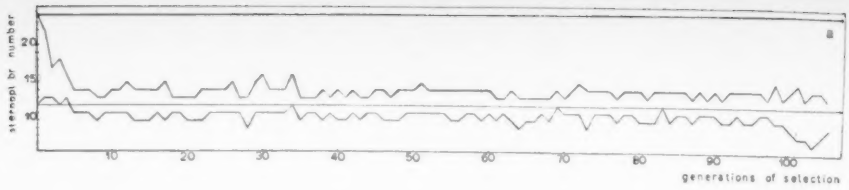
Among the AL-lines, Tg AL was outstanding also in respect of the range. Just as in the SL-lines its low extremes trangressed those of the original population by only a few bristles, whereas the other AL-lines attained bristle numbers far below those of the unselected strains, expecially Ör AL and St AL where even the highest extremes in some generations fell below the original minimum level. Generations with high variance were in these lines characterized by deep falls of the lower limit.

As could be expected, St AH and St AH II which almost completely failed to respond to selection kept well within the original range. The other AH-lines produced extremes above the original maximum limit, although the transgression was small for Ör AH and Alg AH. Tg AH in some of the later generations was wholly above the original range.

The study of the range thus confirms the differences between high and low sternopleural selection and the inconsistency of Tg AL and Tg AH with the other abdominal selection lines.

D. *Reversed and relaxed selection.*

Only some scattered experiments have been undertaken concerning reversed selection from the selection lines. The strength of selection has been the same as in other cases. From Ör SL 51 a line of reversed selection was started. It was continued for 6 generations, but was ineffective in changing the mean (11.82 pooled for all generation, to be compared with 11.84 for Ör SL during the same time). Another reversed selection line was started from Ör SH when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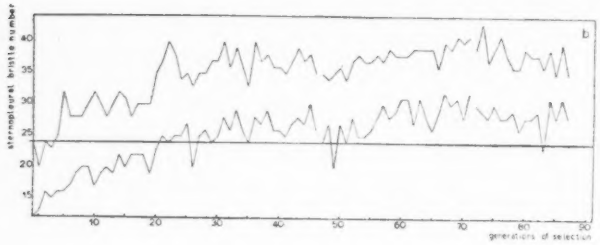
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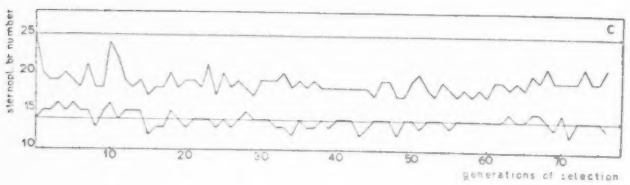
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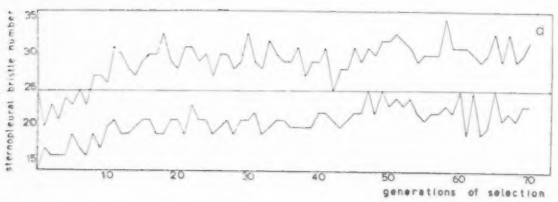
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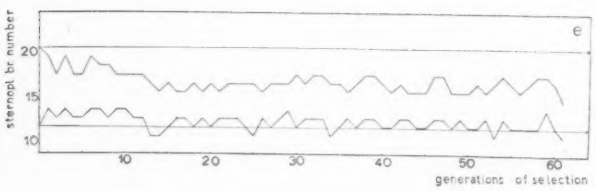
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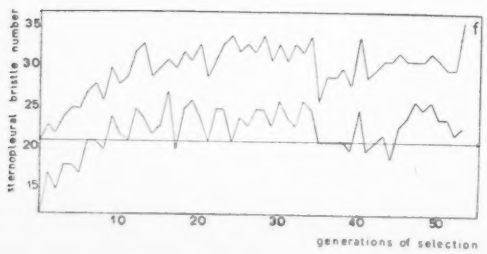
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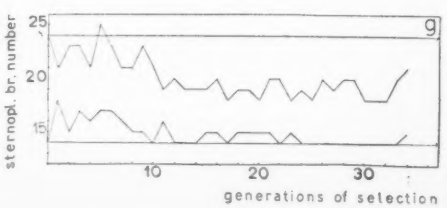
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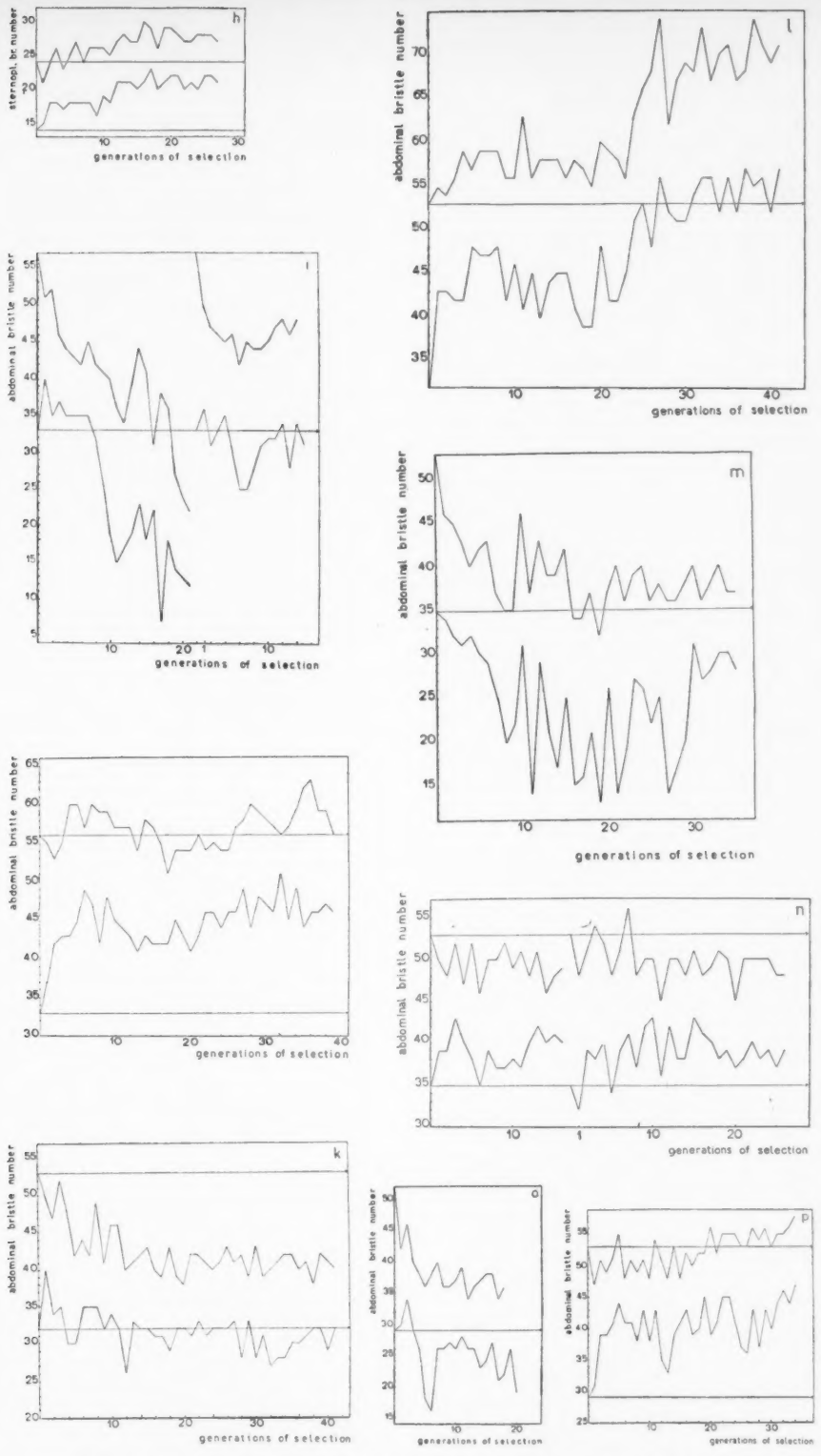

Fig. 5. Changes in the range of bristle numbers during selection. Female values. The range of the unselected strain is in each case indicated by horizontal lines.
a) Ör SL. b) Ör SH. c) Tg SL. d) Tg SH e) St SL. f) St SH. g) Alg SL. h) Alg SH. i) Ör AL and Ör AL II. j) Ör AH. k) Tg AL. l) Tg AH. m) St AL. n) St AH and St AH II. o) Alg. AL. p) Alg AH.

this was renewed from its mass cultured offshoot for the first time. It became extinct after 3 generations. Its mean was 28.35 against 29.23 for the Ör SH-line, thus a little lower. The difference between the generation means is, however, not significant.

The lines Tg SL and Tg SH were subjected to reversed selection, which started from generations 19 and 18 respectively and was continued for 6 generations. For Tg SL the result was a small but significant increase in bristle number (16.72 against 15.78) mostly attributed to the first generation. In Tg SH the reversed selection brought down the mean in each generation and after 6 generations it was as low as 19.82, more than 5 bristles below the simultaneous value for Tg SH. At this stage the lines thus still posessed unfixed variability, especially the SH-line.

When the original selection experiments were considered as finished the selection lines were kept as mass cultures. In every generation 5 pairs of flies were taken out for propagation without selection. Bristle counts were made during the first few generations for informations about the immediate consequences of relaxed selection. The lines were then kept without counts for

TABLE 3. Effects of relaxed selection. The immediate changes are tabulated in the column headed R; N are the values obtained by renewed selection after an uncountrolled period of one year. Where no significant difference was found between S and R, N was compared with the mean of all these generations (last column).

Selection line	Continued selection (S) Mean of 10 generations	Relaxed selection (R)		Renewed selection (N)		Differences		
		Number of gen	Mean	Number of gen	Mean	S—R	R—N	(S+R)—N
Ör SL	11.2	5	10.9	5	11.9	0.3		—0.8***
Tg SL	16.5	6	16.4	2	17.0	0.1		—0.5
St SL	14.4	7	14.1	—	—	0.3		
Alg SL	15.6	4	15.3	5	16.3	0.3		—0.8*
Ör SH	30.7	5	29.7	8	21.9	1.0*	7.8***	
Tg SH	25.1	5	24.1	7	19.6	1.0		5.2***
St SH	26.9	6	26.9	—	—	±0		
Alg SH	23.0	3	21.5	—	—	1.5*		
Ör AL II	35.4	2	35.0	6	36.9	0.4		—1.6
Tg AL	32.7	6	32.2	7	36.1	0.5		—3.6***
St AL	29.8	6	29.9	8	31.0	—0.1		—1.2*
Alg AL	28.7	3	28.2	—	—	0.5		
Ör AH	46.7	5	44.9	6	45.0	1.8		1.1
Tg AH	55.9	5	51.5	6	54.8	4.4**	—3.3	
St AH	41.1	7	39.2	—	—	1.9*		
Alg AH	44.5	5	41.7	4	43.8	2.8*	—2.1	

* Probability 0.05—0.01
** „ 0.01—0.001
*** „ < 0.001

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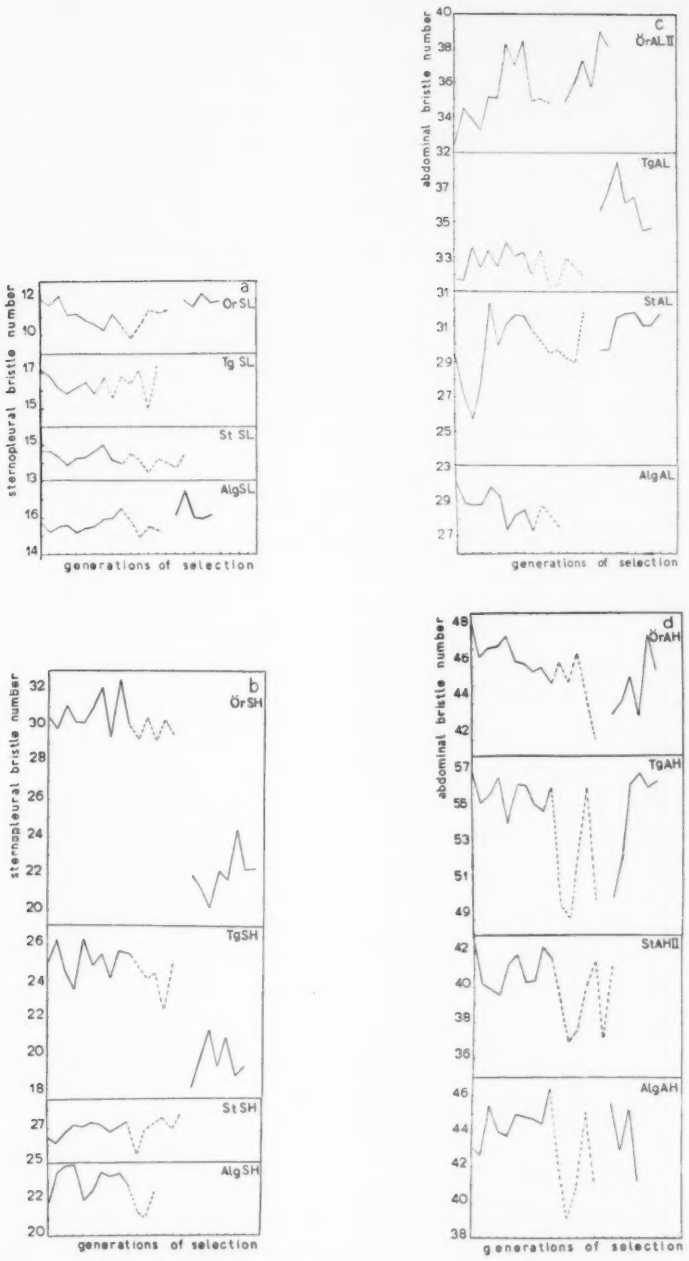

Fig. 6. Relaxed and renewed selection. Generations of relaxed selection are indicated by dotted lines. The time interval between the recorded relaxed selection and the renewed selection is one year.
a) SL-lines. b) SH-lines. c) AL-lines. d) AH-lines.

about a year, or 25—30 generations. During this uncontrolled period some of them died out, but the remaining 11 lines were tested for stability of bristle number at the end of the year. Cultures with 2 × 2 flies were set up from each line, the bristles formerly selected for were counted, and selection started anew for some generations. The results are seen from fig. 6 and from table 3.

In the AL- and SL-lines there are no immediate consequences of the release from selection, except that the newly arisen response in Ör SL disappeared within a few generations, and the line reverted to its former level. During the uncontrolled period a small reversion occurred in all these lines. It was, however, not always significant. The reversion was greatest in Tg AL, where both limits of the range had moved about 6 bristles upwards, but the mean still was well below the original level. In the other lines no definite changes of the range could be found. No line responded to the new selection.

Three of the SH-lines reverted significantly as soon as selection was relaxed. Only the line St SH was not changed. Two lines, Ör SH and Tg SH were left after the uncontrolled period, and both had reverted considerably towards the unselected level. Tg SH had acquired a mean level only one bristle above the original mean of the strain, Ör SH was 5 bristles above its origin. The reduction of bristle number had changed both limits of the range, the new range of Ör SH being wholly below the previous level of the line, but still not quite within the original range, which, however, was the case with Tg SH. The renewed selection was ineffective in raising the bristle number.

All the AH-lines fluctuated considerably when selection was relaxed and a significant reduction of the mean appeared in most of them, even in St AH II, where selection had been almost unable to raise the bristle number. The uncontrolled period had not influenced the lines, for a year later they remained at the same level as when the counts were abolished, and the new selection was able to recover the loss in bristle number in Ör AH and Tg AH (see fig. 6 d) and bring them back to the selection level. In Alg AH the new selection was ineffective, but in this line selection level was attained already in the first generation counted, and in none of the following generations did the mean decrease as much as during relaxed selection.

E. *Interpretation of responses in the selected characters.*

Summing up the changes in sternopleural and abdominal bristle numbers induced by selection, the following results are revealed:

Although the effectiveness of selection varies from strain to strain it can generally be stated that the sternopleural selection is most effective in increasing the bristle number and the abdominal selection in decreasing the number. This is evident from the means but is more accentuated in the ranges, where the SL-lines hardly transgress the limits of the original populations,

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whereas the SH-lines change their range so much that in some lines they become quite separate from the original range. The AH-lines are in this respect most like the SL-lines and the AL- the SH-lines, with the exception of the Tg strain where AH behaves like SH and AL like SL. Reversions against the course of selection extending over several generations are more common in the abdominal selection lines than in the sternopleural ones.

The intraculture variation of the selection lines generally is a little below the level of the original strains, except in the AL-lines where low mean is accompanied by abnormally high variation. Also in this respect Tg AL differs from the other AL-lines in not showing any abnormal variation.

Reversed selection is more effective in SH- than in SL-lines. Relaxed selection causes an immediate reduction of bristle number in SH- and AH-lines. In the AH-lines the reduction is accompanied by an increased fluctuation of generation means. The AL- and SL-lines are not immediately influenced by relaxed selection.

25—30 generations of uncontrolled breeding caused further reversions towards the original level for all except the AH-lines. The reversions were small for the AL- and SL-lines, but very significant for the SH-lines. In no case, however, was the original level quite restored. Renewed selection after this period was ineffective in changing the bristle number, except in 2 of the AH-lines where the loss suffered during the first generations of relaxed selection was regained.

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Consistent results of many selection experiments have confirmed that small phenotypical variation may be associated with great potential genetic variability, that can be released by artificial selection. The question of how the potential variability is hidden so as not to influence phenotypical variation has been answered by MATHER (1941, 1943). He presumes a system of + and — polygenes where each chromosome is balanced, the balance being relational for crossbreeding organisms and internal for self-fertilizers. This system is built up by natural selection for an intermediate optimum by means of crossing over and maintained by means of linkage.

As a consequence of that the balanced system has been acieved through natural selection, MATHER assumes different results of artificial selection, depending on whether selection is exercised on a naturally varying character or on the expressivity of a mutant character. In the former case the response should be slow with periods of stability and response alternating, but selection should in the end bring the range well outside that of the original population, thanks to the breaking up of balanced polygenic combinations. In the latter case there should be an immediate response but no later progress, and no marked transgression of the original limits, because no balanced system has been built up for the mutant character.

MATHER's theory has been criticized (WRIGHT 1945, ROBERTSON & REEVE 1952) on the grounds that he has overrated the importance of linkage. As other contributing factors to the hiding of genetical variability WRIGHT suggests the occurance of locally distributed iso-alleles, and ROBERTSON & REEVE over-dominance and gene systems which under changed genetical or environmental conditions can develop increased effect.

Obviously things are more complicated than a mere balance of + and — genes. This is clear from the present investigation where both types of selection results were attained when selecting for a naturally variable character in opposite directions. The SH-lines gave results of the first type predicted by MATHER, but the SL-lines responded in a way that according to MATHER is typical when selecting for the expression of a mutant character. If the unselected level is assumed to be the optimum maintained by natural selection, this optimum is not midway between the limits of genetical variability but situated near the lower limit. However, the optimum still must have a minimum limit, otherwise natural selection would have brought the bristle number down to zero. This is also implied by the only example of a real decrease below the original range, generations 99—105 of Ör SL, which is associated with a deterioration of fertility.

Reduction of sternopleural number is a rare event, and the discrepancy between the ease of attaining bristle numbers above the original range and the difficulty of reducing the number below it, seems to be caused by lack of genetical variability in the low direction. This is not consistent with the occurance of a balanced system of + and — genes without or with varying direction of dominance, but implies different genetical systems for the increase and the reduction of sternopleural bristle numbers.

Nor can the irregular behaviour of the abdominal selection lines be explained by + and — balance. In the AL-lines of the Örebro, Stäket and Algeria strains the response was immediate and caused a large decrease, but still cannot be attributed to the second of MATHER's types of results, as the range obtained was far below the original one. The correlation found between low bristle number and low fertility together with high variation might suggest genetical heterogeneity. If low bristle number and low fecundity are pleiotropic effects of the same genes, and if these genes are inferior to their alleles that produce higher bristle number, they cannot become wholly prevailing in the population, and thus a high variation might be maintained. This explanation, however, cannot account for the dependance of variation on the bristle numbers of the parents. Parents with low bristle number give offspring with low mean bristle number, a skewed distribution of the numbers, and consequently a high variance, whereas parents with more bristles give offspring with higher mean, a more normal distribution, and a lower variance. The interpretation of this phenomenon is given by the behaviour of the inbred line Ör b, described in a

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previous publication (RASMUSON 1952), and characterized as follows: 1) Inbred by brother-sister mating for 60 generations. No inversions found. 2) Response to selection for abdominal bristles but not for sternopleurals. 3) The response restricted to the first generation and possible to revert in one generation. 4) Low fertility, skewed distribution and high variation in the low selection line.

These facts imply a delayed effect of the parental phenotype that determines the penetrace of a gene reducing abdominal bristle number and fertility; low bristle numbers of the parents giving penetrance less than 50 %, resulting in skewed distribution and high variance; high bristle number giving no penetrance and consequently higher bristle number and lower variance. Whether the dependance is exercised by both parents or by the mother only has not been settled.

The evidence of parent-offspring correlation in apparently homozygous lines is not restricted to this one line. Another line, Ör f, inbred for 100 generations, and with a bristle number quite different from Ör b, was subjected to high and low abdominal selection. The selection was continued for 10 generations, and the difference between high and low lines was found to be 1.43 bristles, significant at the 1 % level. In the last generation reversed selection was also practised and this was effective in changing the position of the means. The result is thus in accordance with that of Ör b as to the expressivity of the character although the difference is smaller, but influence on penetrance is not involved. Another support is the findings of HEUTS (1949) concerning the dependance of fertility on non-genetical variations of abdominal bristle number (see p. 293 RASMUSON 1952). Besides, this seems to be an explanation of the discrepancies REEVE & ROBERTSON find between heritability based on parent-offspring correlation and on the ratio between selection response and selection differential (REEVE & ROBERTSON 1953 a p. 288).

The dependance of the offspring on non-genetical fluctuations among the parents thus appears to be of common occurrance, and explaines the behaviour of the AL lines in the following way: Selection produces a genotype containing in homozygous state at least one gene, the expressivity and penetrance of which is dependent on the phenotype of the parents. This gene is pleiotropic in reducing bristle number as well as fertility. Parents selected for low bristle number give offspring among which the penetrance of the gene is incomplete, causing high variation. When selection is abolished, parents with higher bristle number produce offspring where the gene has no penetrance and variation accordingly is lower. During the course of selection the total genotype is modified so as to decrease the penetrance of the gene in consequence of natural selection for higher fitness. This causes the decrease of variation and the increase of bristle number against the direction of selection, which is found in

St AL and Alg AL. In Ör AL the modifications selected were instead effective in increasing the penetrance, which led to the extinction of the line.

The fluctuations of the AH-lines, extending over several generations, are surprisingly consistent in the 3 lines that responded to selection, Ör AH, Tg AH and Alg AH. All of them increased for 5 or 6 generations, then followed a period of reversion during 8—13 generation, whereafter another increase set in, which however for Tg AH was much faster and more effective than for the others.

The line Alg AH was not simultaneous with Ör AH and Tg AH and so the possibility of seasonal or other environmental fluctuations can be excluded. The explanation must lie in the antagonism between the artificial and the natural selection. The artificial selection picks out genes producing high bristle numbers and rejects those producing low. After 5—6 generations the most effective genes are fixed in the line. When this is achieved modifications become of importance, for the extreme genotypes are not at the optimum, and thus must be rather unfit in the prevailing circumstances. The natural selection, antagonistic to the artificial one, modulates the genetical environment of the bristle increasing genes in order to reduce their effect and thus revert the bristle number towards the original level, in spite of the continuous selection for high bristle number. When the immediate possibilities of modification are exhausted, artificial selection again takes the lead, presumably using new gene combinations produced by crossing over, and a new period of increase follows. In Tg AH a very effective new gene combination must have been produced, for the increase brought the range far beyond the original one. In Alg AH and Ör AH the new advances were smaller, and were in Ör AH followed by another reversion, indicating that the new genotype was again modified by natural selection.

The dependance on the parental phenotype is indicated also for the AH-lines by their response to relaxation of selection. When selection is practised the highest extremes are always chosen as parents, and the offspring maintain the highest possible level. But when no selection takes place the parents may have intermediate or even low bristle number, and the mean bristle number of the offspring must fluctuate much more towards lower numbers. The fluctuations could not be due to changes in the genotype, for new selection after the uncontrolled period brought the mean back to the selection level again.

The genetical environment seems to have greater influence on abdominal bristle number than on sternopleural. Alternation between periods of response and reversions are not common in the SH- and SL-lines, whereas they are found in all abdominal selection lines except St AH and Tg AL, which did not transgress the original range. Possibly the smaller and more numerous abdominal bristles are influenced by genes that are not effective in inhibiting or preserving the development of sternopleural bristles. The difference in ge-

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netical variability towards high and low numbers may be correlated to the space available for the bristles. The abdominal sternites are well defined and restricted areas, and therefore a lowering of the number of abdominal bristles is more likely to occur. The localisation of the sternopleurals is less well defined, which allows variation towards higher numbers. These extra sternopleural bristles are usually small and thus can be assumed to be influenced by smaller genetical changes than are needed to inhibit the development of the normal number. Mutations that increased sternopleural numbers were easily produced by X-rays in the experiments of SCOSSIROLI (1953) and probably are not uncommon under natural conditions also. The marked reversions of the SH-lines during uncontrolled breeding may be ascribed to backmutations of such genes.

II. INDIRECT RESULTS OF SELECTION

A. *The inter-relation between bristle groups on different parts of the body.*

REEVE & ROBERTSON (1953 b) recently pointed out a notable fact concerning the correlation between bristle numbers on different sternites of the abdomen of Drosophila melanogaster. While the genetical correlation between different segments is very high (between 0.9 and 1), they found virtually no environmental correlation. They argue that these bristles are extremely resistant to changes in environment such as humidity, temperature, quality and quantity of food, and conclude that the non-genetical variation must depend on factors acting very locally, and is not of the same kind as the environmental variation usually encountered in dealing with quantitative characters. They call it chance or stochastic variability, and suggest somatic mutations or chance disturbances of hypodermis as possible contributing agencies.

Another similiar indication of the instability of development has been studied by MATHER (1953), viz. the difference between the number of sternopleurals on the left and right side of the fly. Any asymmetry which may appear between sides must be caused by local disturbances, whether arising from differences of environment or upset of cell development.

The occurance of dissimilarities between such homologous areas of the body raises the question of what part local and general factors play on bristle groups on different parts of the body and what the relations are between environmental and genetical correlations. As the selected lines with their great genetical diversity for genes affecting two types of bristles, sternopleural and abdominal bristles, form a convenient material for such an analysis, the following investigation was undertaken:

Four types of bristles were included; besides the two types selected for, the joint number of coxal bristles on the third pair of legs, and the number of frontal bristles on the top of the head, posterior to the ptilinum. Head, thorax,

legs and abdomen were thus represented each by one group of bristles. These groups of bristles were counted on flies from the selection lines and pair cultures from the original strains. The counts were undertaken when the lines were fairly stable, in no case before selection-generation 10 but usually much later (see table 4). The counts were repeated from 2 to 5 times except for line Alg AL where only one count for coxal and frontal bristles was made before the line became extinct. 10 inbred lines from the Örebro strain (Ör a—j) were included as regards sternopleural-abdominal (S—A) bristle correlation, no counts on coxal and frontal bristles being available from these. The origin of the selection lines headed "Temperature experiment" will be accounted for in the next section. In all, counts for sternopleurals and abdominal bristles were available from 44, and for coxal and frontal bristles from 33 different strains and selection lines. As before counts were made on 20 males and 20 females from each culture.

These values have been used for computations of the 6 possible correlations between the bristle types. Two different kinds of estimates were made. In the first case the non-genetical correlations were searched for. The "within culture" correlations of the unselected strains were taken as a proper extimate of these. As there was only one pair of parents in each culture, the intraculture genetical differences must be small. They can, however, not be quite overlooked, and the correlations found must be judged in relation to the genetical correlations.

These were found from the means of male and female means from each strain and selection line, founded on at least 40 individual values (coxal and frontal bristles from Alg AL) and at most 600 (sternopleurals and abdominals from the strain Örebro). They ought to give a fairly reliable estimate of the genetically determined level of the bristle numbers. The standard deviations for culture means within lines were: abdominals 1.696; sternopleurals 0.645; coxals 0.347 and frontals 0.867, and the differences between line means were quite significant even for the unselected bristle types. In most cases the correlations have been computed from the means of only those generations where both types of bristles under consideration have been counted simultaneously. But for the correlations abdominals-coxals (A—C) and abdominals-frontals (A—F) the means from the SH- and SL-lines are not from corresponding generations, nor are the means for sternopleurals-coxals (S—C) and sternopleurals-frontals (S—F) from the AH- and AL-lines. This is, however, not likely to have caused any appreciable error, as the lines were fairly well stabilized when the counts were made. Finally it must be stated, that the counts on the original strains were not made at the start of the selections but at later occasions, and it is possible that changes have occured in their genetical composition during the interval. No such changes were, however, indicated in a previously undertaken analysis (Rasmuson 1952).

The correlations found are seen from table 5. They are all positive, but

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TABLE 4. Generations of simultaneous counts of different bristle types.

Line or strain	Abdominal bristles		Sternopleural bristles		Coxal+Frontal bristles	
	Number of counts	Gen. during which counts were performed	Number of counts	Gen. during which counts were performed	Number of counts	Gen. during which counts were performed
Selection lines						
Ör						
SL	5	53—69			3	92—96
SH	5	58—66			3	76—81
AL			5	11—16		
AL II			5	11—15	3	11—13
AH			5	11—15	3	25—31
Tg						
SL	5	29—38			3	62—65
SH	5	28—37			3	57—61
AL			5	10—14	3	24—31
AH			5	27—32	3	25—30
St						
SL	5	16—26			3	49—53
SH	5	15—24			3	45—48
AL			5	14—21	3	23—27
AH II			5	11—15	3	16—19
Alg						
SL	5	12—16			3	17—23
SH	5	11—15			3	16—19
AL			5	16—21	1	32
AH			5	12—16	3	17—23
Temperature experiment						
Ör						
SL untr.	2	14—15			2	14—15
SH „	2	15—16			2	15—16
Tg						
SL „	2	15—16			2	15—16
Alg						
SL „	2	15—16			2	15—16
SH „	2	15—16			2	15—16
ASO, all lines	2	20—21			2	20—21
Inbred lines						
Ör a-j	5	8—20	5	8—20		
Original strains						
Örebro	15		15		5	
Tunnelgatan	10		10		5	
Stäket	10		10		5	
Algeria	10		10		5	

TABLE 5. Correlations between bristle types. The correlation coefficients are in I based on intraculture correlations of individual bristle number, in II on correlations between means of selection lines. Significances are designated as in table 3. S = sternopleural, A = abdominal, C = coxal, F = frontal bristle number.

I. Non-genetical correlations.

Type of correlation	Unselected strains Degrees of freedom 379				Alg SH and Alg SL Total correlations	
	Females		Males		Females	Males
	Total	Partial	Total	Partial	D. of f. 334	D. of f. 316
S—A	0.3198***	0.3026***	0.6103***	0.6144***	0.9543***	0.5315***
S—C	0.0902	0.0518	0.0811	0.0727	0.0447	0.1481*
S—F	0.1507**	0.1174*	0.1090*	0.0855	0.0638	—0.0374
A—C	0.1234*	0.0986	0.0332	—0.0210	0.0395	0.0071
A—F	0.1233*	0.0870	0.0604	—0.0027	0.0295	—0.0373
F—C	0.0376	0.0165	0.0537	0.0456	0.1192	0.0443

II. Genetical correlations.

Type of correlation	Degrees of freedom	Total	Partial
S—A	42	0.4399**	0.0609
S—C	31	0.6289***	0.5223***
S—F	31	0.6932***	0.5399***
A—C	31	0.2804	0.0945
A—F	31	0.5593***	0.3938*
F—C	31	0.4093*	—0.0811

not all significant. As the correlations may influence each other, the partial correlations when the two other bristle groups are held constant, are also given in the table. Only two significant values are then left among the non-genetical correlations, S—A and S—F. The agreement between males and females is good, but the S—A correlation is significantly stronger for males.

The genetical correlations are all positive, and, except for A—C, significant. This contradicts the assumption that the indirect responses of selection should be due to accidental linkage conditions, and suggests that real physiological relations and pleiotropic gene action occur between bristle groups on different parts of the body. The computation of partial correlations, however, leaves only two strong correlations, S—C and S—F, and one suggestive, viz. A—F. There is no correspondance to the strong S—A correlation from the individual values, and this must therefore be caused by environmental influences.

A more penetrating analysis of the interrelations between bristle groups

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can be made, following a method given by S. WRIGHT (1932) for partitioning the variation of related measurement on general, group and special factors. Least-square fitted path coefficients are computed which give a maximum estimate of determination by general factors. Appearance of significant residuals suggests the existence of group factors and the specific factors are responsible for that part of the variation which is not accounted for by the correlations.

This method yielded the results seen from table 6. As regards the non-genetical correlations, no group factors were indicated. The coxal bristles did not give any significant correlations with other bristle types, and was therefore excluded from the analysis, the special factors consequently being put at 100 %. The correlations from the male values turned out to give a path coefficient for sternopleurals greater than 1, which of course is absurd, but follows from the method of computation giving the maximum estimate of dependance by general factors. The determination of sternopleurals by the general factor was therefore given as 100 %.

The genetical correlation could be separated in 3 different systems of group factors, besides the general and the specific factors. One group included abdominal and frontal bristles, a second group sternopleural and coxal bristles and a third, which however did not give significant values, sternopleural and frontal bristles.

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The group factors from this analysis thus corresponds to the partial regressions found, but the factorial analysis also gives percental estimates of these and of the general and specific factors. The physiological phenomena that can account for these relationships are not easily seen. It will be necessary to give a brief survey of the development of the imaginal hypoderm, this being the tissue that produces the bristle forming cells. The following informations are taken from ROBERTSON (1936), LEES & WADDINGTON (1942) and BODENSTEIN (1950).

The imaginal hypoderm of head, thorax and legs is formed from the anterior parts of the corresponding imaginal discs, starting from their connections with the larval hypoderm. The imaginal hypoderm of the abdomen is, for all segments except the last one, formed from hypodermal histoblasts that appear between the larval epidermis cells and gradually replace them.

Of the 4 concerned bristle types, the *frontal* bristles are formed from hypoderm originating from the eye-antenna-disc, the *sternopleural* bristles originate from thoracic hypoderm from the second leg discs, the *coxal* bristles from the third leg discs, and the *abdominal* bristles from the hypodermal histoblasts of the 4th and 5th abdominal segments.

Two factors that clearly may be of importance are the original position and the time of development for the tissues producing the different bristle groups.

In the mature larva, the discs for eye-antenna and for first and second leg

TABLE 6. Partitioning of the variation on general, group and special factors.

I. Non-genetical variation.

Bristle type	Females					Males				
	% of total variance		Actual variance		Coeff. of variation	% of total variance		Actual variance		Coeff. of variation
	General factors	Special factors	General factors	Special factors		General factors	Special factors	General factors	Special factors	
Sternopleural	39	61	1.11	1.74	9.49	100	0	3.69	0	10.96
Abdominal	26	74	2.66	7.58	7.31	34	66	2.44	4.73	7.34
Coxal	0	100	0	1.10	6.64	0	100	0	1.21	7.05
Frontal	6	94	0.17	2.72	14.27	1	99	0.03	3.09	15.22

II. Genetical variation.

Bristle type	% of total variance					Actual variance					Coeff. of variation
	General factors	A—F group factors	S—C group factors	S—F group factors	Special factors	General factors	A—F group factors	S—C group factors	S—F group factors	Special factors	
Sternopleural . .	73		9	4	14	64.54		7.96	3.53	12.38	18.98
Abdominal . . .	28	21			51	36.11	27.09			65.78	7.68
Coxal	32		23		45	0.97		0.70		1.37	6.93
Frontal	57	12		6	25	9.13	1.92		0.96	4.00	21.58

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pairs lie close to each other, and are all connected with the ventral ganglion; whereas the haltere, wing and third leg discs are more laterally situated, and are connected to the main tracheal trunk. The hypodermal histoblasts of the abdomen are well separated from all these discs. The space relations thus place the frontal and the sternopleural bristles in one group and indicates no association between the others.

The time relations for developmental events are in outlines as follows:

96	hours after haching from the egg:	puparium formation.
98	„ „ „ „ „ „	on thorax, imaginal hypodermal cells grow into the larval hypoderm.
100	„ „ „ „ „ „	abdominal histoblasts appear.
101	„ „ „ „ „ „	larval hypoderm replaced ventrally and laterally on thorax.
102	„ „ „ „ „ „	legs everted.
103	„ „ „ „ „ „	imaginal hypoderm of thorax completed.
108	„ „ „ „ „ „	pupation; eyes and antennae everted.
111	„ „ „ „ „ „	bristle-forming cells appear on thorax.
126	„ „ „ „ „ „	bristle-formation begins „ „
130	„ „ „ „ „ „	imaginal hypoderm of abdomen completed.

The imaginal hypoderm is thus completed on thorax before the head is everted and shorly after the abdominal histoblasts have begun to appear. At the same time the legs are everted. This seems to place the sternopleural and the coxal regions earlier in sequence than the frontal and abdominal regions, and might account for the correlations between on one side sternopleurals and coxals and on the other side frontal and abdominal bristles.

These considerations do not furnish any proof of the sequence of bristle determination, and such evidence is apparently very difficult to produce. LEES & WADDINGTON found that 111 hours after haching, bristle forming cells on the dorsal part of thorax were distinct from other epidermis cells. This fixes the latest time for bristle determination at about 8 hours after completion of the corresponding hypoderm, but influences may be induced at much earlier stages. This is indicated by some experiments with high temperature treatment during limited periods of development, undertaken by the author. These experiments gave no information about the sequence of determination of the 4 bristle types, but the temperature sensitive period for bristle number in general was found to be confined to the larval and prepupal stage, as treatment during the 4 last days of development was without influence. HENKE and collaborators (HENKE, v. FINCK & MA 1941) have studied the induction of modifications by temperature chocks during different stages of development, and have located the sensitive period for bristle modifications (reduced, forked,

stunted, and missing bristles) to the third larval instar, the puparium, and the earliest part of the pupal stage. However, single modified individuals appeared also after temperature treatments during earlier stages. This is all that at present is known about the time of bristle determination, and lacking further information, the time sequence of bristle determination may be assumed to correspond to that of hypoderm formation.

According to this hypothesis, the S—F correlation is thus explained as being due to correspondance in position of the origins and the S—C and A—F correlations as being due to correspondance in the time of determination. It may be assumed that bristle producing substances are regulated from a common center, for instance the ring gland. This might connect the bristle production of the frontal and sternopleural regions, the imaginal discs of which are adjacent and close to this gland, and cause the S—F correlation. Further, the accessibility of substances is likely to fluctuate during development, and this may account for the correlation between the simultaneously determinated groups of bristles S—C and A—F.

It is necessary to consider both space and time to account for the relationships between bristle groups. In other cases one has to assume several bristle influencing centra at different places, and even then it is difficult to explain the A—F correlation that unites the most anterior with the most posterior bristle group. As only 4 groups of bristles are included, the analysis cannot be brought any further. This should require an extension of the material to many groups of bristles including more body segments, and also a closer study of the development of the hypoderm.

It remains to account for the strong correlation found between sternopleural and abdominal bristles in the analysis of intraculture correlations. It cannot be explained by remaining genetical variation as might be the case with the S—F correlation, for there is no genetical S—A correlation. The existence of a correlation that is not genetically conditioned, proves that non-genetical variation cannot be wholly attributed to "stochastic variation" because the correlation must be caused by some more generally acting factor. There is no correspondance of position or time of determination between these groups of bristles, so any agency that influences sternopleural and abdominal bristles, but not coxal and frontal bristles, is most likely to act on the superficial parts of the body during development, as the spreading of thoracic and abdominal hypoderm takes place at the surface whereas the legs and the head develop as sacs within the body and later evert. Humidity might be suggested as a possible agency.

The evidence of the environmental S—A correlation was further strengthened by computation of within culture correlations from the two selection lines Alg SH and Alg SL. The values originated from an experiment on temperature influences and the cultures were submitted to a temperature of 31° C during

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certain stages of development. The material included 355 females and 336 males. The intraculture correlation coefficients are given in table 5. The S—A correlation is found to be even stronger than in the unselected strains. It is possible that the high temperature during development has accentuated the variation of the environmental factor and thus increased the correlation.

With the exception of the S—A correlation the non-genetical variation is mostly locally determined, which is in accordance with REEVE's & ROBERTSON's calculations on sternites. The local determination is complete for the coxal bristles and almost so for frontal bristles. (The influence found of common factors on the frontals may be due to remaining genetical variation.) The dependance of local and general factors is in no way correlated with the magnitude of the variation, which measured by the coefficient of variation places the bristle types in the following order: F > S > A > C (see table 6).

Compared with the non-genetical variation, a greater part of the genetical variation depends on common factors. Most locally determined as regards the genetical variation are the abdominal bristles, which might be presumed because of their position, remote from the other bristle groups.

The genetical variation cannot be directly compared for the unselected and the selected bristle types, and a coefficient of variation was therefore computed, where only those lines were included that had not been subjected to selection for the bristle type in question. The sequence turned out to be the same as for the non-genetical variation: F > S > A > C (see table 6). There thus seems to be a close relationship between the variability between individuals caused by instability in development or environmental influences and the capacity to express phenotypically differences in the genotype. The cause may be the simple fact that the more stable bristle types on the coxal and abdominal segments are confined to more distinct and well defind areas.

The inter-relations between mean bristle numbers (the values for computation of the genetical correlations) are shown in fig. 7. The 4 types of selection lines, the unselected inbred lines and the original strains are differently marked, and it can be seen that the lines of a special type sometimes form a group separated from the rest. This is most clearly shown by the SH- and AL-lines on fig. 7 a, the relationship between sternopleural and abdominal bristle numbers. Not only are all these lines transferred towards higher sternopleural bristle numbers (or lower abdominal bristle numbers) but within themselves they form a separate regression line. The total S—A correlation is according to table 5 0.4399 (d.f 44) but when the AL- and SH-lines are excluded it becomes 0.7189 (d.f 29), thus definitely stronger. Within these two groups taken together the correlation is as high as 0.9116 (d.f 15).

The correspondance between the AL- and SH-lines suggests that some common developmental mechanism is acting when abdominal bristle number is reduced and sternopleural number is increased. Perhaps this mechanism upsets

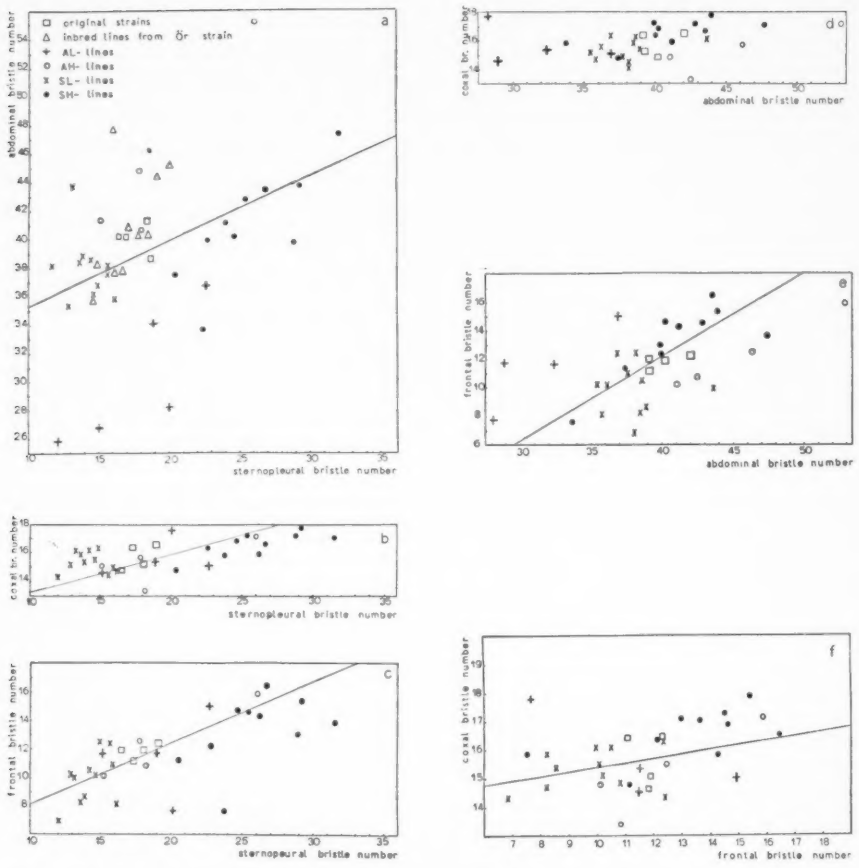

Fig. 7. Correlated responses to selection. Each point is the mean of male and female means from 1—15 different cultures or generations (see table 4). In cases of significant correlation the regression line is drawn.

a) The relation between sternopleural and abdominal bristle numbers.
b) The relation between sternopleural and coxal bristle numbers.
c) The relation between sternopleural and frontal bristle numbers.
d) The relation between abdominal and coxal bristle numbers.
e) The relation between abdominal and frontal bristle numbers.
f) The relation between frontal and coxal bristle numbers.

the regularity of bristle patterns, thus affording opportunities for the formation of extra sternopleural bristles and the inhibition of abdominal bristles. Besides this mechanism, selection has influenced the more general capacity of bristle production, which is shown by the positive correlation between the number of abdominals and sternopleurals within the two groups.

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TABLE 7. Correlated bristle responses in opposite lines of selection from a common origin. Significances of the differences are designated as in table 3. The double sternopleural values of low abdominal bristle selection in the Örebro strain are derived from Ör AL and Ör AL II. Coxal and frontal bristles were only counted on Ör AL II.

	Response of	Common origin	Mean bristle number			Differences		
			H High sel.	O Origin	L Low sel.	H—O	O—L	H—L
Sternopleural bristle selection	Abdominal bristles	Örebro	47.4	40.1	38.1	7.3***	2.0	9.3***
		Tunnelgatan	41.2	41.4	37.6	—0.2	3.8**	3.6**
		Stäket	43.5	40.3	38.6	3.2*	1.7	4.9**
		Algeria	33.7	38.6	35.8	—4.9**	2.8*	—2.1
	Coxal bristles	Örebro	17.1	16.4	14.3	0.7*	2.1***	2.8***
		Tunnelgatan	15.9	16.5	14.9	—0.6*	1.6***	1.0*
		Stäket	16.6	14.7	16.1	1.9***	—1.4**	0.5*
		Algeria	15.9	15.2	14.7	0.7*	0.5	1.2*
	Frontal bristles	Örebro	13.7	11.1	6.9	2.6**	4.2***	6.8***
		Tunnelgatan	14.3	12.3	10.8	2.0*	1.5	3.5**
		Stäket	16.5	11.8	10.5	4.7***	1.3	6.0**
		Algeria	7.6	11.9	8.2	—4.3***	3.7**	—0.6
Abdominal bristle selection	Sternopleural bristles	Örebro	17.9	16.8	12.3 22.7	1.1*	4.5*** —5.9***	5.6*** —4.8***
		Tunnelgatan	26.0	18.4	18.9	7.6***	—0.5	7.1***
		Stäket	15.2	16.4	15.2	—1.2*	1.2*	0
		Algeria	18.2	18.6	20.1	—0.4	—1.5**	—1.9**
	Coxal bristles	Örebro	15.6	16.4	15.1	—0.8*	1.3**	0.5
		Tunnelgatan	17.2	16.5	15.4	0.7*	1.1**	1.8**
		Stäket	14.8	14.7	14.6	0.1	0.1	0.2
		Algeria	13.4	15.2	17.9	—1.8***	—2.7***	—4.5***
	Frontal bristles	Örebro	12.5	11.1	15.0	1.4	—3.9***	—2.5*
		Tunnelgatan	15.9	12.3	11.6	3.6**	0.7	4.3**
		Stäket	10.2	11.8	11.6	—1.6*	0.2	—1.4*
		Algeria	10.8	11.9	7.7	—1.1	4.2***	3.1*

B. *Comparisions between opposite lines from the same origin.*

Table 7 gives the correlated bristle responses in opposite lines of selection from each of the four strains Örebro, Tunnelgatan, Stäket, and Algeria. The levels of the original and of the derived means follow in most cases the same sequence as the selected means, H > O > L, which agrees with the positive correlations found between the bristle types. In some cases, however, the sequence is reversed, high selection lines having reduced, and low selection lines having increased the means of the unselected bristle types. This is most

common in the Algeria strain where the SH-line has significantly lower number of abdominal and frontal bristles than the unselected strain, the mean values being even less than in the SL-line. Further, the AH-selection from this strain has reduced the numbers of the unselected bristles (though the differences are insignificant for sternopleurals and frontals) and the AL-selection has increased the number of sternopleurals and coxals.

Reversed sequences of bristle means are also found in some other strains. SH-selection from strain Tunnelgatan has reduced the number of coxal bristles and SL-selection from strain Stäket has increased the number of coxal bristles. Ör AL II has a very high number of sternopleural and frontal bristles whereas the coxal number is reduced. St AH II did not differ significantly from the unselected strain as regards the selected bristle type but still there is a significant reduction of sternopleural and frontal bristles.

Two possible explanations of this phenomenon are evident. Either it may be due to accidental linkage between locally acting genes that influence the bristles in opposite directions, or it may be due to selection of more generally acting genes that induces a competition between the bristle groups. If linkage is assumed, the probability of getting no correlated response should be greater than the probability of getting a reversed response, if the locally acting genes are supposed to be randomly distributed over the chromosomes. But the cases of insignificant changes are 14 and of significant reversed responses 13, so this consideration affords no decision between the two possibilities. It is, however, to be noted that the reversed responses are most pronounced between abdominals-frontals on one side and sternopleurals-coxals on the other. Thus the reductions in Alg SH are more striking for abdominals and frontals than for coxals and the increases in Alg AL and Ör AL II are limited to sternopleurals and coxals. This is reflected in the group factors that relates abdominals with frontals and sternopleurals with coxals, as previously discussed. Such a deviation from randomness favours the competition hypothesis, as it is not possible to explain by accidental linkage between locally acting genes.

These results may be compared with those from Mather's and Harrison's selection experiments (Mather & Harrison 1949, p. 147—150). They have counted the number of sternopleurals and coxals of 11 lines selected for abdominal bristle number and their unselected origins. For the sternopleurals they found a relation best fitted by a parabola, i.e. the lowest and the highest abdominal numbers were accompanied by high sternopleural numbers. Their two low selection lines are almost identical in abdominal as well as in sternopleural bristle numbers. They indicate that there has been competition between the bristle types as the sternopleural numbers are higher than in the original strains. The position of line 3, which has been selected for high number of abdominals but following relaxed selection reverted to the original level, shows, that in spite of the complete reversion of abdominal bristle number the geno-

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type is changed so that the sternopleural level is lower than before. Lines 4—9 have all originated from line 3, and among them increases in abdominal numbers are in all cases accompanied by increases in sternopleural number. Here selection has acted on the general bristle level. The selection from the original cross thus accomplished a competition between abdominals and sternopleurals both in the high and the low line. It led to reduction of fertility, and in the high line the infertility was so severe that selection had to be relaxed. The abdominal level then reverted to its original value and fertility improved, but the low sternopleural level persisted, indicating that the competitive gene system had not been lost. The new selection from line 3 acted on other genes, influencing the general bristle level, and the result this time was as high or higher than the first reached, without the accompanying loss of fertility. The reversed selection, represented by the lines 7—5—2, has again been acting by competition, increasing the sternopleural number while reducing the abdominal one.

The coxal numbers demonstrate further that the increase in lines 4—9 has been accomplished by generally acting genes. Line 3, however, has not changed the coxal number in relation to the origin, and also the low selection line 2 has a coxal number close to the origin. The competition found between abdominals and sternopleurals has not affected the coxal bristles, and therefore most likely has acted on the differences in position and not in determination time.

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The correlation system outlined above can thus also account for the results of Mather's and Harrison's experiments, undertaken with other strains and under conditions other than those of the present investigation.

III. ATTEMPTS TO INCREASE SELECTION RESPONSE BY EXAGGERATION OF THE CROSSING OVER FREQUENCY

The importance of linkage conditions for the outcome of selection has been stressed by MATHER, but is denied, or at least doubted, by ROBERTSON & REEVE (1952, p. 444). The possibility of testing the importance of linkage is indicated by PLOUGH's findings in 1917, that crossing over is more common in certain parts (especially the proximal ones) of the second and third chromosomes, when the flies are subjected to extreme temperatures, low as well as high. This increase is manifested in the progeny emerging from eggs laid on the sixth and seventh day after the temperature treatment. A corresponding increase does also follow after X-ray treatment according to MULLER (1925).

It can be supposed that at least some of the bristle determining genes are located within the sensitive chromosome segments. It should then be possible to demonstrate whether the response to selection is influenced by changes in

the frequency of crossing over. In choosing between temperature and X-ray treatment, the former was preferred, as X-rays are more apt to induce mutations during the course of the experiment and thus upset the results.

An experiment involving the 4 strains Örebro, Tunnelgatan, Stäket and Algeria was outlined as follows:

From each strain selection for high and low number of sternopleural bristles was undertaken in the usual way with 2 × 20 animals examined and 2 × 2 selected in each generation. The selected animals were exposed to a temperature of 31° C for 48 h, and were then placed in 25° C. 5 days from the start of the temperature treatment the animals were transferred to new culture bottles, and after another 5 days they were discarded. The offspring from the eggs laid during the last 5 day period are most likely to show exaggerated crossing over and were accordingly used for the selection in the next generation. This treatment was repeated in every generation. The mating of the selected animals took place before the temperature treatment, to which therefore also the males were exposed, although they do not show any crossing over under normal conditions.

Simultaneously, control lines were run in exactly the same way as the treated lines, with the exception of the high temperature treatment. To get the identity in origin of treated and untreated lines as close as possible, the separation took place in the first generation so that the flies emerging from eggs laid during the first 5 days, before the effect of temperature treatment had set in, were used for the control lines.

The outcome of this experiment is shown in fig. 8. No consistent difference in total response could be found between treated and untreated lines. The treatment thus has not had any pronounced effect on the final level which selection can attain. However, the diagrams show that the response was more rapid throughout in the treated lines of high selection, whereas no difference could be found between treated and untreated lines of low selection. The interpretation is, that genes determining high number of sternopleurals are linked within the sensitive chromosome parts, linkage is loosened by the temperature treatment so that advantageous combinations can be produced more readily than under the usual conditions, but obviously the same combinations are also produced sooner or later without treatment. The failure of the treatment in the low lines must be due to the special conditions that characterize this kind of selection. As discussed earlier, selection for low sternopleural bristle number seems to act only on the genotypes already present in the original population, sorting out the unsuitable ones without producing any new combinations. Under such circumstances crossing over cannot influence the effectiveness of selection.

To establish that the differences found were really due to increased crossing over and not to other influences of the temperature treatment, the experi-

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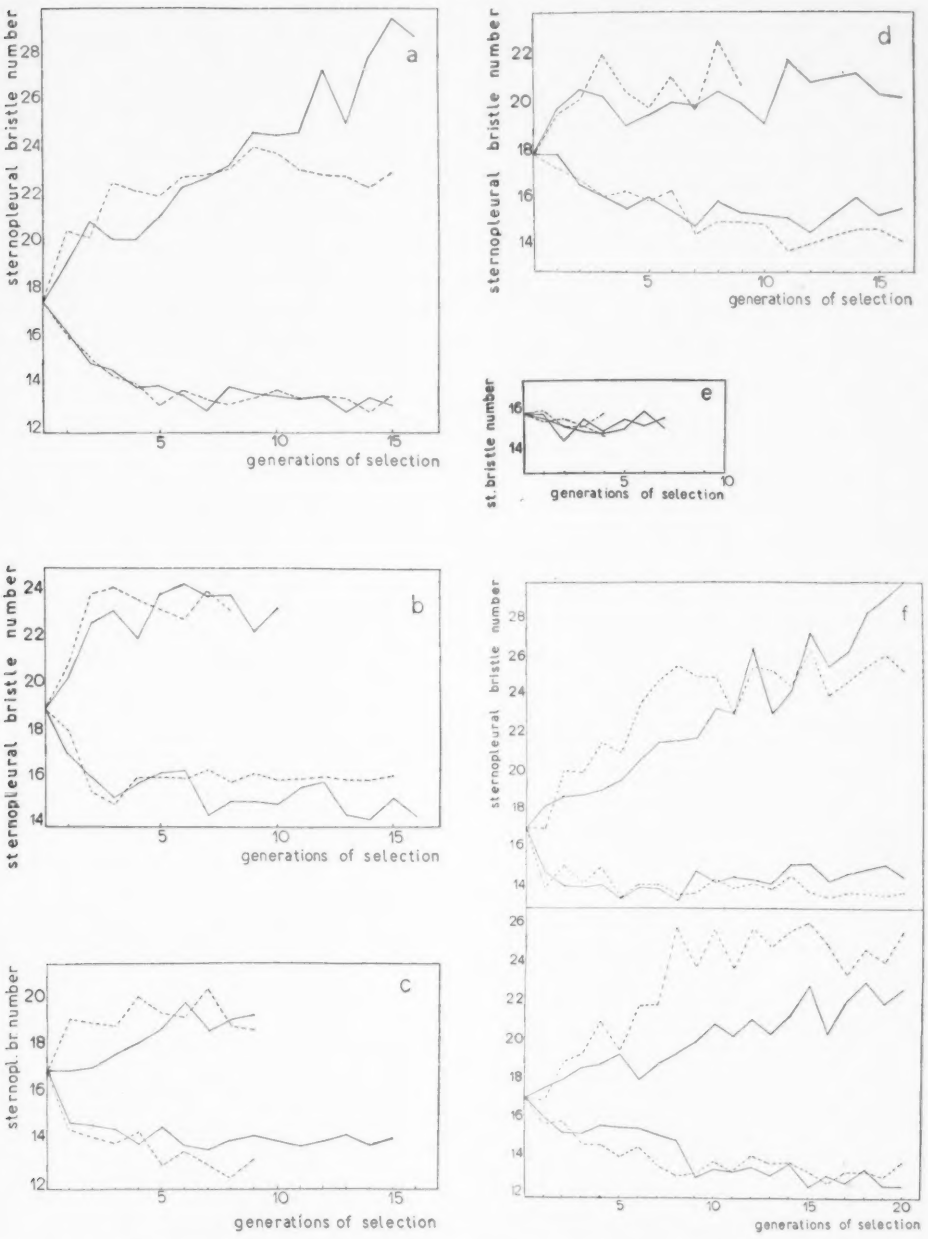

Fig. 8. Effect of temperature induced exaggeration of crossing over frequency on the responses to sternopleural selection. — Untreated lines. - - - - Treated lines.
a) Selection from strain Örebro. b) Selection from strain Tunnelgatan. c) Selection from strain Stäket. d) Selection from strain Algeria. e) Selection from inbred Florida line. f) Parallel selections from a pooled Algeria—Stäket—Oregon (ASO) population.

ment was repeated with a long inbred line of the Florida strain (fig. 8 e). Because of the inbreeding the line was very sensitive to the temperature treatment, and selection could only be maintained for 4 generations in the treated lines. The control lines were kept for 7 generations. During this time there was no response to selection in any direction, which proves that the genetical variability was exhausted by inbreeding. Nor was there any difference between treated and untreated lines. The differences found in the other strains must thus be judged as due to different frequency of crossing over, because mutations and modifying influences of the temperature treatment would be as active in a homozygous as in a heterozygous population.

A pooled mean square was computed for the high selection lines and another for the low lines, giving a standard deviation for differences between means for high lines = 0.4545 and for low lines = 0.2848. Mean differences exceeding 1 bristle are thus significant.

The strains from which these selection lines were started had at that time been cultured in the laboratory for a maximum of 4.5 years and a minimum of 1 year and some of their potential variability might have been lost. If the strains had become more or less homogeneous, selection may have had only a few genes left to work on in the sensitive parts of the chromosomes, and then the role of crossing over might be underestimated.

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Another experiment was therefore undertaken, where selection was started from a population attained by pooling 3 laboratory strains, Algeria, Stäket and Oregon, (called ASO), to ensure a high degree of heterozygosity. This experiment was outlined as before, with a few exceptions. Firstly, the number of parents was increased to 2×3 instead of 2×2 in each generation to reduce the chances of genetical drift. Secondly, the temperature treatment was only continued for 12 generations, whereafter all lines were cultured at 25° C. It was thought that temperature treatment itself might be an obstacle to selection, as 31° C is near the general limit of endurance for the flies. It is possible that some gene combinations, favourable to selection, are unable to live or propagate in this temperature, which thus increases the stress of the counteracting natural selection. Thirdly, the experiment was doubled so that 2 treated and 2 untreated lines were run in each direction. The lines on the upper part of fig. 8 f (experiment I) originated from the most extreme males and females of the flies examined in the original population, the lines on the lower part of the figure (experiment II) from the next most extreme ones.

These experiments confirm the former results, only more pronounced, presumably because of the greater heterogeneity of the population. The treated high lines were both more rapid in their responses to selection. After 10 generations the untreated lines in experiment I had drawn level with its corresponding treated line and later on passed it. The high untreated line II approached the treated line from generation 9—17 but never quite reached it.

The low lines in experiment I run much alike but in the end the treated line reached a lower level than the untreated. This last gain may be due to the cessation of the temperature treatment, for thereafter the mean of the treated line was slightly lower. The treated line II was ahead of the untreated during generation 3—9 but after that the lines were inseparable. This experiment thus indicates that crossing over may also be of importance for the low selection.

The double experiments I and II may be used also for illustrating the similarity that can be attained by parallel selections. The treated high lines are very much the same in their whole appearence, whereas the untreated high lines after running together for the first 5 generations are widely separated, line I exceeding line II by about 5 bristles. In view of the similarity between the experimental lines this can hardly be ascribed to the inferior initial position of line II but rather to genetical drift and chance assortment.

Contrary to the high lines there is a pronounced difference between the low lines of experiment I and II. In experiment I the response to selection is rapid, both lines having reached a minimum level already in generation 5 and gaining very little after generation 2. In experiment II the decrease continues until generation 9 where the untreated line overtakes the treated line. Their level is then lower than that reached in experiment I and even later there is a small decrease at least in the untreated line. The difference in behaviour between the low lines of experiment I and II may be interpreted as follows: In experiment I the best genotypes are picked out already in the first selection made, and in the next few generations accompanying unsuitable genotypes are sorted out until the minimum level is reached. Experiment II is started with less extreme genotypes, and consequently segregation and crossing over in the following generations may produce genotypes better fitted to the demands of selection. The response is slow, in fact there is no advance between generation 2 and 7 for the control line, but the temperature treatment shows its accelerating capacity and the final level is lower than in experiment I. In the low lines the less severe selection in the beginning is thus an advantage, whereas in the high lines it does not seem to make any difference to the final result.

IV. OUTCROSSES OF SELECTION LINES AND RENEWED SELECTION FROM THE CROSSBRED OFFSPRING

In the foregoing chapters, it has been shown, that although the magnitude of the selection response varies, some similiar changes are induced in different populations when subjected to the same kind of selection. These similarities involve the range of the SL-lines, the variation of the AL-lines, and several cases of correlated responses in other bristle types. Observations of this kind

raise the question of whether, or to what extent, selection assembles identical genes in unrelated populations.

The only way to solve this problem is by outcrossing. When many genes are involved and there is interaction present, the results from crosses may, however, be confusing. More information may be obtained if renewed selections are started from the crossbred offspring and the end results of selection are recorded.

Outcrosses of two different kinds have been performed: crosses between selection lines of the same type but from different origin and crosses between selection lines and inbred unselected lines from the same origin.

The first attempt to analyse gene differences was a cross between two SH-lines which is recorded in fig. 9. One of the lines was Ör SH, which after gen. 30 on 3 different occasions was crossed to a line calles XSH. This line has not been mentioned previously because its origin was obscure. It was started from an inbred Florida strain, which however was contaminated at an early stage of selection. At the time for the crosses it had been stabilized for many generations on a level between 21 and 23 bristles, thus much lower than Ör SH. 5 crosses were made, three with mothers from Ör SH, two with mothers from XSH. From the figures it is seen that the F_1 in 4 cases coincided with the lower parent; in one case it was intermediate. Selection for high bristle numbers was started from 4 F_1-populations, two of each reciprocal cross, the 5th F_1 was subjected to reversed selection. The high selection lines gave different end results. ÖrX I reached the level of the high parent after 5 generations and was stabilized there; XÖr II stayed on a level 2—3 bristles above the XSH-level and ÖrX II about 1 bristle above this. XÖr I was the only line which showed an increase over the highest parental line. The reversed selection line declined about 1—2 bristles below the level of XSH. This reduction should be compared with the results of reversed selections from the parental lines, started at about the same time (see fig. 9 e). It seems to be a little larger than the sum of these, but apparently is not as great as the transgression obtained in the opposite direction.

Coincidence between F_1 and the less extreme parent is to be expected if it is assumed that the genes responsible for the extreme bristle numbers are recessive to those maintaining the normal level, and the two selection lines have assembled some identical genes. The one exceptional case of intermediate F_1 points either at instability of the dominance relations or at genetical heterogeneity of the lines. The end results of the high selection lines prove by the transgression that a more effective genotype can be obtained by combinations of the bristle-increasing genes from the two lines. That the transgression obtained in the opposite direction is smaller may depend on metric bias. Two of the end results were intermediate but on different levels, and indicate that the higher mean level of Ör SH must depend on at least 3 gene differences.

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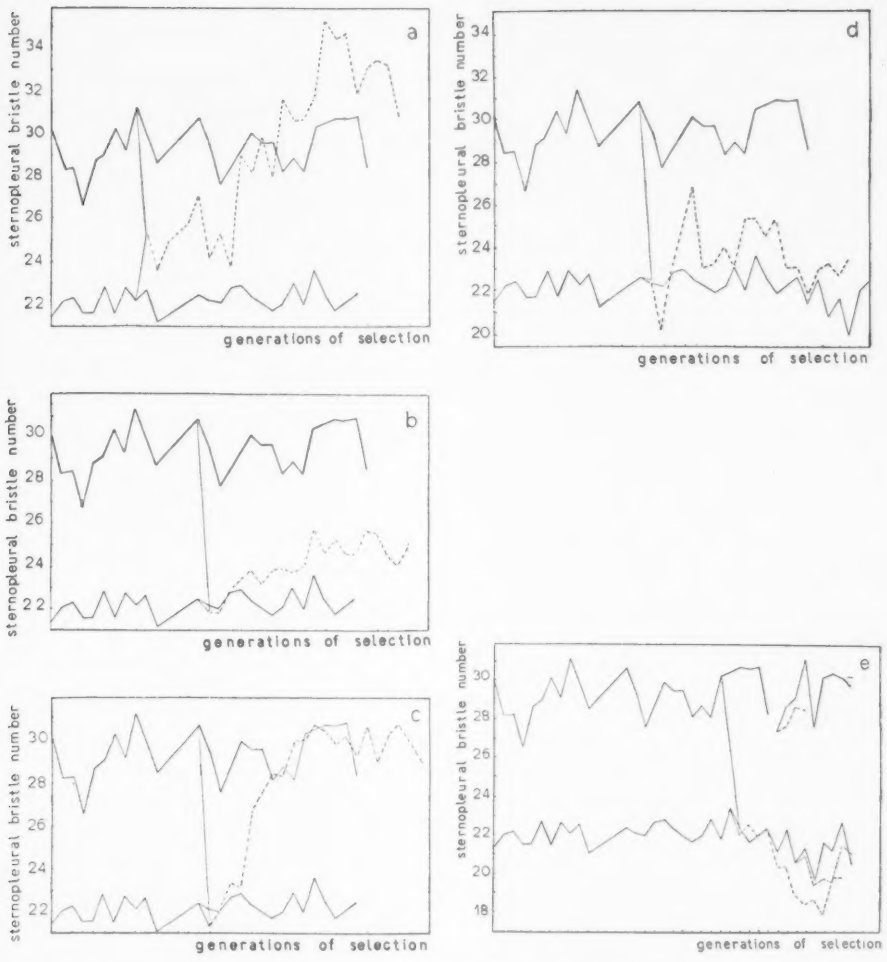

Fig. 9. Sternopleural selection from crosses between Ör SH and XSH. — Parental lines. ---- Selection from crossbred offspring ------ Retrogressive selection from parental lines.
a) XSH × Ör SH I. b) XSH × Ör SH II. c) Ör SH × XSH I. d) Ör SH × XSH II. e) Retrogressive selection from Ör SH × XSH.

The minimum assumption of non-identical genes influencing bristle number must thus be 4 to account for the results of the crosses.

The second outcrossing experiment involved the SL-lines. The crosses Ör SL × St SL, Ör SL × Tg SL, Ör SL × Alg SL and Alg SL × St SL were made, selections for low bristle number were started from F_2 and continued for 8—10 generations. As distinguished from the SH-crosses all the F_1:s were intermediate between the parental lines and the end results of selection

were all identical with the lowest parental line, no intermediate and no transgressing results being obtained.

Crosses between selected lines and fixed genotypes of less extreme kinds were performed with Ör SH and Ör SL which were crossed to 3 different brother-sister inbred lines of the Örebro-strain, Ör b, Ör g and Ör j. The crosses were made between single individuals from generations 39—43 of Ör SL, generations 34—37 of Ör SH and generations 40—50 of the inbred lines. Two or three parallel crosses were made with the inbred flies as mothers. They gave consistent results and their pooled F_1- and F_2-values are shown in fig. 10 together with the selections made from the crosses.

The F_1 from Ör j × Ör SH gave a mean bristle number of 18.4 and the range kept well within the range of the original Örebro strain. The F_1 from the cross Ör j × Ör SL was inseparable from Ör j itself. Renewed selection from F_1 brought the lines back to their extreme levels in a few generations but did not bring them any further.

The crosses to Ör b and Ör g gave consistent results. The F_1:s from the crosses to Ör SH were intermediate between the parental lines and their range transgressed that of the original Örebro population. The F_1:s from the crosses to Ör SL gave a mean of about 16 bristles and kept within the unselected range. Selections from F_1 of the Ör b crosses restorted the bristle numbers of the selection lines. From the crosses to Ör g no selection was undertaken.

As the inbred lines were not selected, they must be supposed to have fixed random mixtures of the genes available in the original population. Of the inbred lines used in the crosses, Ör j had accumulated genes causing a low number of bristles whereas the other two had rather high bristle numbers. None of them did, however, transgress the range of the original population. The bristle numbers of the F_1:s give information about the genetical consistency between the selected and the inbred lines. When Ör SH was crossed to Ör j the mean of the F_1 was at the unselected level, but the F_1 from Ör j × Ör SL was at the level of Ör j. This indicates that the extreme bristle numbers of Ör SH were caused by recessive genes, the normal alleles of which were present in Ör j. Likewise the low level of Ör SL depended on recessive genes, but some of these were also present in Ör j, and the cross between these lines was not able to restore the unselected bristle mean. On the other hand, the restoration was possible in the crosses of Ör SL to Ör b and Ör g, presumably because these lines were able to afford the dominant alleles of the bristle reducing genes in Ör SL. Ör SH was not brought back to the unselected level by outcrossing to Ör b and Ör g, which suggests that Ör SH, Ör b and Ör g possess some identical genes for increased bristle number.

The intermediate position of the F_1:s from Ör SH × Ör b and Ör SH × Ör g does not, however, fit in with the hypothesis of recessivity of genes causing extreme bristle numbers. A possible explanation is a change of dominance

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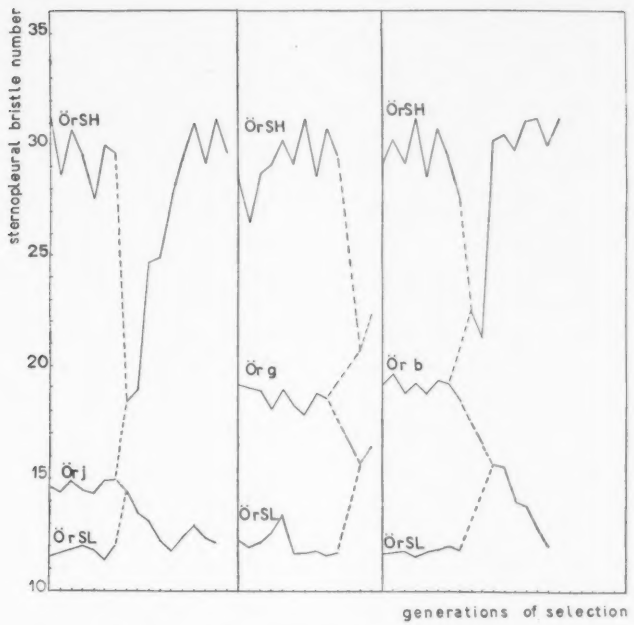

Fig. 10. Outcrosses of Ör SH and Ör SL to inbred lines from the same origin, and renewed selection from the crossbred offspring.

relations caused by the selection for extreme numbers, a change which is maintained in outcrosses to individuals which themselves are extremes of the natural range in the same direction.

The restoration of selection levels is easily obtained after these outcrosses and after the crosses between SL-lines. This suggests that few genes with distinct phenotypical effects are segregating. The failure to obtain transgressions indicates that no more effective new combinations are produced. This is contrary to the SH-crosses where a transgression could be obtained, but restoration of the highest parental level was not achieved in all cases. It is obvious that the opportunities for new gene combinations are greater in the SH-crosses.

Three important interpretations may thus be drawn from the results of the outcrosses: The genes responsible for extreme bristle numbers are recessive to the genes for normal bristle numbers, but may among themselves show varying dominance relations. Lines derived from the same origin have partially identical genotypes, and this is also indicated for the SH-lines from different origin. More factors are segregating in the crosses between SH-lines than in crosses between SL-lines and between related lines.

DISCUSSION

As the usual Mendelian methods are inapplicable in the study of quantitative inheritance, the number and localization of the individual genes involved in the determination of a certain character cannot be directly determined. Yet knowledge of these things is essential for the understanding of the importance of linkage conditions. Assuming that the loci are distributed resonably evenly on all chromosomes, in a species like Drosophila with only 4 pairs of chromosomes, linkage conditions must already be of importance for about 10 loci, whereas in species with high number of chromosomes, linkage can hardly be of significance unless 50—100 loci are involved. If on the other hand, the genes influencing a certain character are not randomly distributed, the importance of linkage is greatly exaggerated.

Mather confines the polygenes to the heterochromatic parts of the chromosomes, and this might give rise to some clustering. In his calculations on abdominal bristle number in Drosophila, he was not able to establish the existence of more than one segregating factor in crosses between high and medium lines and two in crosses between low and medium lines, which is interpreted as a result of linkage (MATHER & HARRISON 1949, p. 39). Further examples of clustering of genes influencing the same character are cited by SHEPPARD (1953) who points out that in polymorphic species the genes responsible for the polymorphism are often linked. The linkage may vary from what appears to be multiple alleles up to crossing over values around 20 %. He suggests that the linkage has been evolved as a result of natural selection following different selective values of different gene combinations. His examples cover color patterns in insects, snails and fishes, and blood groups in man and cattle.

It seems possible that the same process has also been at work for the bristle determining genes. The three important criteria, polymorphism, linkage and different selective values, can all be found in the present data.

That the natural populations are polymorphic as regards bristle determining genes is evident. It appears from the behaviour of the SL-lines, where a certain part of the original bristle range is isolated by means of selection. Parallel inbreeding experiments from a common original strain also separate into lines with different ranges of bristle number (RASMUSON 1952). This of course does not characterize only bristle numbers but is a common trait for many quantitative characters, which may be said to show hidden polymorphism.

The results of crossing over exaggeration by means of temperature increase give evidence of linkage between sternopleural bristle increasing genes, but with one exception not between genes reducing the sternopleural bristle number. The linkage found in the high lines did not seem to be very close, because it was easily broken up even without any exaggeration of the crossing over frequency. It is possible that the almost complete failure to find evidence of

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linkage between the bristle reducing genes may instead be interpreated as close linkage, which is seldom broken up. This assumption would explain both why no transgression of the original range could be obtained in the SL-lines, and why the selection levels were so easily restored after outcrosses of these lines. The whole linkage group would act as a unit, and the number of such units would of necessity be small. It may also explain the intermediate F_1:s of the SL-crosses, for if interaction is present, such complex factors must give an "aggregate dominace" (following the nomenclature of ROBERTSON & REEVE 1953) or "potence" (following WIGAN, 1944) which is not likely to be complete. It is to be expected that interaction between genes at different loci is stronger when the loci are closely linked than when they are more remote, and therefore may be of importance in the SL-crosses although it is not evident in the other crosses.

The evidence of linkage is fetched exclusively from the sternopleural selection. There is, however, nothing which contradicts that the conditions are generally the same for the abdominal bristles. But the consistency is greater within the SL- and SH-types of selection, and further, the delayed non-genetical effect of the parental bristle number on the offspring, and the modifying influences of the total genotype on the responses to abdominal bristle selection make the influences of linkage conditions less clear in this case. The linkage appears to be closer between genes that increase the abdominal bristle number than between those that reduce the number of these bristles, because the original range is more readily transgressed in the low direction than in the high.

The last requirement, that of different selective values for different gene combinations, is fulfilled by the poor fertility found in some of the selection lines. The infertility is usually most pronounced in lines where the range of the original population is considerably transgressed; for instance in generations 99—105 of Ör SL, in Ör SH and St SH, and in the AL-lines of the Örebro, Stäket and Algeria strains. This agrees with the qualification that the unselected range must be at the optimum. Exceptions are the poor fertility of Tg SL, which does not transgress the original range, and the good fertility of Tg AH, which does. The first exception may be explained by accidental fixation of deleterious genes linked to the bristle reducing factors. In the second case one may point at the consistent results of MATHER and HARRISON (1949) who also attained in their experiments high number of abdominal bristles without reduction of fertility, whereas their low selection lines could not be maintained because of infertility. It thus seems as if high number of abdominal bristles would not reduce the fitness under laboratory conditions, which, however, does not prove that such individuals could compete with the normal type in natural populations.

The bristle groups on the abdominal sternites and the sternopleural areas

are common to many Diptera and presumably are of very ancient origin. The characteristic numbers vary between species. During the course of evolution, bristle determining genes may have been brought close together and linked in such positions as to make the most advantageous combinations the most frequent. The linkage has become more or less close in different gene systems, and the closeness determines the behaviour of the system under artificial selection for an extreme optimum.

Perhaps a connexion may be found between the varying linkage conditions and the mutability of the partaking genes or their importance for fitness, but more penetrating investigations are demanded before anything can be stated about this problem. It ought also to be mentioned that there are no proofs of whether the bristle number in the present case is itself the adaptive character or whether it is only a secondary effect of pleiotropic genes with other more important but as yet unknown effects. This is, however, not essential for the interpretations of the results.

SUMMARY

1. 4 newly raptured wild strains of Drosophila melanogaster were selected for high and low number of bristles on the sternopleural areas and on the 4th and 5th abdominal sternites. 2 wild type laboratory strains, which had been cultured for at least two decades, were selected for high and low sternopleural bristle number.

2. All the wild strains responded to selection, except in one case of high abdominal bristle selection. The maximum increase of sternopleural bristle number was 111 %, of abdominal bristle number 36 %. The maximum reduction was 36 % for sternopleural and 59 % for abdominal bristle number. In the laboratory strains a separation between the high and the low line amounting to at most 1—2 bristles was obtained.

3. The intraculture variances of the selection lines usually decreased below those of the unselected populations. 3 of the AL-lines showed in some generations an abnormally high variation accompanied by reduced mean bristle number and low fecundity.

4. The range of phenotypic expression was changed by the selection, and transgressed in all the SH- and most of the AL- and AH-lines the limits of the unselected populations. In the SL-lines, selection was unable to reduce the bristle numbers below those already present in the unselected populations, except in one case of late response.

5. Reversed selection was effective after about 20 generations of sternopleural selection in one strain, but not after 50 generations in another. The influence of relaxed selection was studied at the end of the selection. The

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immediate consequences were a reduction of about 1 bristle in the SH-lines and increased fluctuations towards lower generation means in the AH-lines. The AL- and SL-lines were not changed. The lapse of 1 year (= 25—30 generations) without selection caused a reversion almost to the unselected level in the two remaining SH-lines, and a small but significant increase in the AL- and SL-lines. Renewed selection was ineffective. The AH-lines were not changed during the uncontrolled period, and renewed selection brought the mean back to the selection level.

6. The behaviour of the selection lines is discussed in relation to MATHER'S theory of balanced systems of polygenes, and it is concluded that a + and — balance cannot account for the results obtained. Instead, it is suggested that different genetical systems are responsible for the increase and the reduction of bristle number. More segregating factors seem to influence the increase than the reduction of sternopleural bristle number.

7. The high intraculture variation in the AL-lines is explained by a delayed effect of the parental phenotype on the penetrance of a gene causing low abdominal bristle number. Other examples of similiar phenomena are given, and it is shown that influence of the parental phenotype is also exercised in the AH-lines. Long term fluctuations in the abdominal selection lines are interpreted as modifying influences of the genetical environment, which is modulated by natural selection.

8. Genetical and non-genetical correlations were computed for 4 types of bristles: sternopleural, abdominal, coxal and frontal bristles. The non-genetical correlations were computed from the individual bristle numbers of the unselected strains and of late generations of Alg SH and Alg SL. Only one significant non-genetical correlation was found, viz. between sternopleural and abdominal bristle numbers. The genetical correlations were computed from the means of late generations of the selection lines and their unselected origins. The correlations found were all positive, which indicates a pleiotropic influence of the bristle determining genes. Following a method indicated by S. Wright, the variation was divided into parts depending on general, group and special factors. The non-genetical variation was mostly locally determined, and indicated no group factors. The genetical variation was more dependent on general factors and 3 systems of group factors were indicated, one correlating sternopleurals and coxals, one sternopleurals and frontals, and one abdominals and frontals. A survey of the development of the imaginal hypoderm is given, and it is suggested that the sternopleural-frontal correlation is caused by the close initial position of the imaginal discs producing the bristle forming tissues, whereas the correlations sternopleurals-coxals and abdominals-frontals are caused by simultaneous determination of the bristles. The non-genetical correlation between sternopleural and abdominal bristle numbers prove that the individual fluctuation in bristle number are not wholly locally determined.

9. Selections in opposite directions from the same origin usually caused correlated responses of other bristle types in corresponding directions. Some cases of reversed responses were found. It is assumed that this depends on competetive gene action rather than on accidental linkage of locally acting genes.

10. High temperature treatment was used in order to exaggerate the crossing over frequency and hereby influence the responses to sternopleural selection from the 4 wild strains and a pooled population from 3 unrelated strains. The end results of selection were not generally improved by the treatment, but the responses were accelerated in the high selection lines, proving that the genes for increased sternopleural bristle number are linked.

11. Crosses were performed between 2 SH-lines and between 4 SL-lines from different origins. Ör SH and Ör SL were crossed to inbred, unselected lines from the same original strain. The mean bristle level of the F_1:s and the end results of selections from the cross products indicated that the genes causing extreme bristle numbers were recessive to those maintaining the normal numbers, and that identical genes had been fixed in lines originating from the same strain and in the SH-lines from different strains. The crosses confirmed that more factors were segregating in the SH- than in the SL-line crosses.

12. Varying linkage conditions, evolved by aid of natural selection, are suggested as a possible explanation for the different behaviour of the selection lines.

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GENETIC AND CYTOLOGICAL STUDIES OF DROSOPHILA FUNEBRIS

SOME SEX-LINKED MUTATIONS AND THEIR STANDARD ORDER

BY

ANN-MARGRET PERJE

(Institute of Genetics, University of Stockholm, Stockholm, Sweden)

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I. INTRODUCTION

N. W. TIMOFÉEFF-RESSOVSKY (1931) published a linkage map of some sex-linked mutations of *Drosophila funebris*. LÜERS (1938) gave some further crossing-over results in the same species. In this paper crossing-over experiments with nine different sex-linked mutants of *D. funebris* are reported. These data are discussed in connection with the linkage data of TIMOFÉEFF-RESSOVSKY and LÜERS. Moreover they show much similarity to the linkage data given for *D. virilis* by CHINO (1936).

In order to determine the free end and the centromere end of the X-chromosome, cytological investigations of the salivary gland chromosomes of some mutations are carried out.

N. W. TIMOFÉEFF-RESSOVSKY (1936) has pointed out that there are qualitative and quantitative differences between the mutability of *D. funebris* and that of *D. melanogaster*. Quantitative mutations with incomplete penetrance and low expressivity are more common than all-or-none mutations like those affecting body colour or eye colour. The same phenomena are described by PERJE (1954). Experiments on the mutation rate in the *yellow, vermilion, singed* and

white loci after irradiation, reported in this paper, show that these genes mutate at about the same rate as in *D. melanogaster* and in *D. virilis*. Thus the differences between *D. funebris* and those species will not depend on a lower mutation rate of the eye colour and body colour mutations as indicated by N. W. TIMOFÉEFF-RESSOVSKY, but rather of the bristle and wing mutations with incomplete penetrance being more common.

The three types of experiments described below, the crossing-over experiments, the cytological investigations of the salivary glands, and the X-ray experiments with the *y v sn w* mutations, illustrate the main part of the X-chromosome of *D. funebris* to be a homologue of the original X-chromosome of other Drosophila species, such as *D. virilis* and *D. melanogaster*.

II. METHODS

Breeding methods described by PERJE (1954) are used. In the crossing-over experiments pair matings are usually employed, but sometimes crosses are made with two females and three males. If not otherwise stated, data from cultures with sex-linked lethals are rejected. Usually three or more loci are segregated in the crossing-over experiments. The results from the individual experiments are not given but data are pooled from all experiments involving a particular combination of mutations. Results from crossing-over in coupling and repulsion are given separately.

The salivary gland preparations were produced and examined under the same microscopic conditions as described by PERJE (1954).

The X-ray experiments were made in the following way: wild type males from an L stock, derived from a single pair mating from the L strain, were irradiated with about 3240 r in a Skandia Intensiv apparatus (170 kV, 15 mA with a filtration equivalent to 2 mm Al), and crossed to females from a *y v sn w* stock. Every culture was made from 2 females and 3 males and the mutants from these crosses were progeny-tested.

The wild type strains used were the same as described by PERJE (1954).

III. DESCRIPTION OF MUTATIONS

car, carnation. Spontaneous recessive sex-linked mutation in the *sc ev* stock. A similar mutation has not previously been described. The eye colour is darker than the mutation with the same name in *D. melanogaster* and it does not correspond to any of the known eye colours of that species. It is lighter than the wild type colour of *D. funebris*. Penetrance is complete. In combination with *vermilion* it gives a light colour very similar to honey in *D. melanogaster*.

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ev, eversae. (N. W. TIMOFÉEFF-RESSOVSKY, 1931). The mutation used here is obtained from the L strain after X-raying. It is a recessive with up-turned wings and usually 100 % penetrance.

m, miniature. (H. A. TIMOFÉEFF-RESSOVSKY, 1930). One male was obtained after irradiating females from the P strain. It is sex-linked recessive with small and dark wings. Penetrance is complete. Homozygous females are sterile. The stock soon became extinct.

N, Notch. (N. W. TIMOFÉEFF-RESSOVSKY, 1931 and DIS 2). The *N* mutation used in these experiments was obtained after X-raying of strain L males. Unlike the *N* mutation described by TIMOFÉEFF-RESSOVSKY, the penetrance is complete. It is a dominant sex-linked mutant, with notches at distal ends of the wings and in homozygous condition is lethal.

sc, scute (PERJE, 1954). A spontaneous recessive mutation from the L strain, usually with only the two post scutellar bristles. Penetrance is more than 95 % and expressivity variable.

sn, singed. (N. W. TIMOFÉEFF-RESSOVSKY, DIS 2). This mutant is the same as described by KIIL in DIS 20 as sn^2. Penetrance is complete.

v, vermilion. (LÜERS, DIS 5). Recessive with light red eyes. Obtained from X-irradiation of the E strain (PERJE, 1954). The eye colour is not as light as *vermilion* in *D. melanogaster.* A later mutation with the same eye colour from the B strain was shown to be an allelomorph.

w, white. (N. W. TIMOFÉEFF-RESSOVSKY and LÜERS in DIS 5, PERJE, 1954). Sex-linked recessive with the same phenotypic effect on eye colour, ocelli and larval and adult Malphigian tubes as *white* in *D. melanogaster.* It arose in the L strain after X-raying.

y, yellow. (PERJE, 1954). A recessive sex-linked from the P strain. Body yellow, but bristles and hairs black. Penetrance complete.

IV. VIABILITY TESTS

The viabilities of six sex-linked mutants are given in table I. Heterozygous females in pair cultures were used in order to avoid overcrowding. The data from the recombination tests were added if no other mutant gene segregated within a distance of 30 crossing-over units from the mutant in question. All cultures with sex-linked lethals were rejected. The estimation of the viability is made as by SPURWAY (1945) in *D. subobscura.*

In *ev* there are significant differences between the sexes. In *sc* the viability is calculated in males only, as a percentage of the heterozygous females show semi-dominance. The other four mutations tested seem to be equally viable in both sexes, thus the combined data of males and females are given in table I.

TABLE I. Viability of some sex-linked mutations of *D. funebris*

mutation	sex	total showing mutant A	total showing wild type B	viability $\frac{A}{B} \pm \sqrt{\frac{A(A+B)}{B^3}}$
cv	♀♀	2 881	2 097	1.374 ± 0.039
ev	♂♂	5 292	4 672	1.133 ± 0.023
sc	♂♂	4 612	4 727	0.976 ± 0.020
sn	♀♀ + ♂♂	6 928	7 376	0.939 ± 0.016
v	♀♀ + ♂♂	3 849	3 812	1.010 ± 0.023
w	♀♀ + ♂♂	17 522	18 208	0.962 ± 0.010
y	♀♀ + ♂♂	8 415	8 502	0.990 ± 0.015

One to five percent of the *sc* males show no penetrance of the *scute* effect probably depending on modifiers. These flies will lower the viability value.

Table I shows that *y* and *v* have the same viability as the wild type, and that *w*, *sn*, and *sc* are detrimentals. The viability of the *ev* mutation is remarkable in that both males and females are more viable than the wild type. This mutation was named *eversae* because of phenotypic similarities to a sex-linked mutant described in the same species by N. W. TIMOFÉEFF-RESSOVSKY (1931). In tests with this mutation TIMOFÉEFF-RESSOVSKY (1939) found that the viability was higher than in the wild type allelomorph at a temperature of 25° C. This being the temperature employed in all these experiments, the mutation used here is possibly an allele of that found by TIMOFÉEFF-RESSOVSKY. The higher viability value in the females is probably due to the character showing penetrance in some percentages of the heterozygotes.

V. GENE ORDER AND MAP DISTANCES

Gene order and map distances in a chromosome are mainly determined by estimating the recombination values between adjacent mutations. If the linked loci are closely spaced the recombination value may itself be used as measure of the distance. The map distance between two loci more widely separated from each other is greater than the recombination value depending on the occurrence of multiple crossing-over and interference. As this map distance cannot be directly observable by genetical methods, the recombination values between neighbouring genes will serve as map distance in this investigation. Thus the genetical chromosome length will increase with the location of new mutations between the mutations known.

Recombination values between eight different sex-linked mutations are given in table II. In cases where estimates of both coupling and repulsion phases are available the differences between recombination values are not significant. The combined recombinations from the coupling and repulsion are calculated from the formula of FISHER, described by SPURWAY (1945).

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TABLE II. Recombination percentages of eight sex-linked mutants of *D. funebris*.

sex	region a	region b	recombination data: in coupling ab/++ ++	ab	a+	+b	recombination %	in repulsion a+/+b a+	+b	ab	++	recombination %	combined recombination
♀♀	*sc*	*y*	1 179	1 106	96	147	9.61 ± 0.59	—	—	—	—	—	—
♂♂	*sc*	*y*	1 296	1 123	11	145	6.06 ± 0.47	591	583	48	13	4.94 ± 0.62	5.47 ± 0.40
♂♂	*sc*	*v*	—	—	—	—	—	1 644	1 790	400	497	20.71 ± 0.62	—
♀♀	*sc*	*ev*	977	1 676	1 120	1 205	46.71 ± 0.71	—	—	—	—	—	45.7 ± 1.6
♂♂	*sc*	*ev*	1 223	1 368	1 056	1 210	46.65 ± 0.72	61	77	59	53	44.8 ± 3.1	
♂♂	*sc*	*w*	—	—	—	—	—	1 135	1 121	1 053	1 173	49.67 ± 0.75	—
♂♂	*y*	*v*	130	130	36	42	23.1 ± 2.3	1 653	1 672	374	401	18.90 ± 0.61	20.9 ± 1.1
♀♀+♂♂	*y*	*sn*	1 759	1 673	1 626	1 530	47.91 ± 0.61	94	82	72	90	47.9 ± 2.7	47.9 ± 1.4
♀♀	*y*	*N*	—	—	—	—	—	368	362	333	370	49.1 ± 1.3	—
♀♀+♂♂	*y*	*w*	2 077	2 027	1 973	1 944	48.83 ± 0.56	124	136	125	152	51.6 ± 2.2	50.2 ± 1.1
♂♂	*v*	*ev*	—	—	—	—	—	315	332	243	196	40.4 ± 1.5	—
♂♂	*v*	*sn*	—	—	—	—	—	102	84	70	82	45.0 ± 2.7	—
♂♂	*ev*	*w*	—	—	—	—	—	1 260	1 076	743	692	38.05 ± 0.79	—
♀♀+♂♂	*sn*	*w*	2 348	2 194	1 009	1 037	31.06 ± 0.57	141	162	73	76	33.0 ± 2.2	32.0 ± 1.1
♂♂	*m*	*w*	—	—	—	—	—	64	73	18	30	25.9 ± 3.2	—
♀♀	*N*	*w*	—	—	—	—	—	678	723	17	15	2.23 ± 0.39	—
			++	a b *and* +b		a+		a+	+b *and* a b		++		
♀♀+♂♂	*v*	*w*	1 735	3 231		1 622	48.32 ± 0.86	1 497	2 863		1 383	48.02 ± 0.93	48.17 ± 0.63

The discrepancies in the *sc y* males where one of the crossover groups is much smaller than the other in both the coupling and repulsion could not be explained only by assuming it to depend on lethals, as the sex ratio was normal in all the pair cultures. If the recombination value is calculated from the two crossover groups combined, it will be about 5 %. If calculated only from the smaller recombination group, it would be not more than about 1 %. GIRVIN (1949) has remarked on the influence of *yellow* mutations (in experiments with variegated *yellow*) on the scutellar bristle number in *D. virilis*. Variegated *yellow* flies which carry an X-chromosome broken just to the left of the *scute* locus, often show doubling of some of the scutellar bristles, but flies which are hyperploid for the left end of the X show a reduction in the number of bristles. In some cultures up to 30 per cent of the flies are affected, but in others only 1—2 per cent. Flies of this class usually have two or three scutellar bristles. The unexpected results in the crossing-over experiments with *y* and *sc* in *D. funebris* could depend both on interaction between the two mutations and on crossing over. In such a case there can be no reliable estimate on the map distance between the two loci until they can be crossed to some other gene in the neighbourhood. No such mutation has been detected. In any case the two mutations *y* and *sc* must lie near each other in the left end of the X-chromosome. The *y* stock itself shows no reduction of the scutellar bristles.

When considering recombination between *v* and *w* it is seen that one of the non-recombinant and one of the recombinant classes cannot be distinguished from each other as *v w* flies show white phenotype. The estimation of the recombination is made only on the other non-crossover and crossover class.

The gene order is determined with 3-, and 5-point tests. The 3-point tests in table III give the following gene orders

$$sc - y - v - ev - N - w \text{ and } y - sn - w.$$

N is only tested against *y* and *w*. As the distance is so long between $y - N$ and $y - w$ it is not quite clear whether *N* is to the left or to the right of *w*.

A 5-point test with the newly found mutant *car* and the *y v sn w* stock gave the results accounted for in table IV. This cross showed that the combinations

TABLE III. Results of three-point tests for gene order in the X-chromosome of *D. funebris*.

loci in the three-point test			region of recombination			
			0	I	II	I and II
sc	*y*	*ev*	373	19	335	17
sc	*y*	*v*	1 996	113	423	43
sc	*ev*	*w*	942	817	590	505
sc	*v*	*ev*	518	129	439	87
y	*N*	*w*	710	691	20	12
y	*sn*	*w*	142	78	152	34

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TABLE IV. Result of a five-point test for gene order in the X-chromosome of *D. funebris*.

$$\frac{\begin{matrix} I & II & III & IV \\ y & v & + & sn\ w \end{matrix}}{+\ +\quad car\quad +\ +} \times \frac{y\ v\ +\ sn\ w}{\quad /}$$

♂♂											
a = phenotype	*y sn w*	*sn w*	*y w*	*w*	*car*	*y car*	*y v car*	*y v*	*y v sn*		
b = recombination					0	I	II	III	IV		
c = number of flies	560	491	241	241	483	137	272	145	209		
♂♂											
a	*v car*	*v*	*v sn*	+	*sn*	*car sn*	*y*	*y sn*	*y car sn*	*y v car sn*	*v car sn*
b	I+II	I+III	I+IV	II+III	II+IV	III+IV	I+II+III	I+II+IV	I+III+IV	II+III+IV	I+II+III+IV
c	43	47	75	37	151	33	2	16	9	14	0

sex ratio = O,487

recombination in interval

I	19.67 ± 0.97 %
II	32.0 ± 1.1 %
III	17.15 ± 0.92 %
IV	30.3 ± 1.1 %

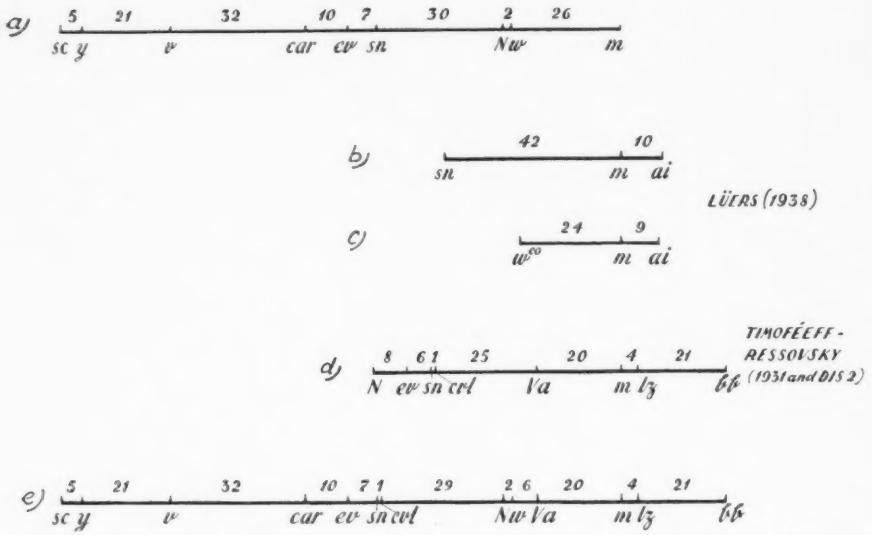
a)
5 21 32 10 7 30 2 26
sc y v car ev sn Nw m
b)
42 10
sn m ai
LÜERS (1938)
c)
24 9
w^co m ai
d)
8 6 1 25 20 4 21
N ev sn cvl Va m lz bb
TIMOFÉEFF-RESSOVSKY (1931 and DIS 2)
e)
5 21 32 10 7 1 29 2 6 20 4 21
sc y v car ev sn cvl Nw Va m lz bb

Fig. 1. A genetical chromosome map of *D. funebris*. a) the map constructed from the investigations in this paper. e) the map constructed with the aid of the combined results from a, the recombination values from LÜERS (b and c) and from TIMOFÉEFF-RESSOVSKY (d). — *ai* = alae incompletae, *bb* = bobbed, *cvl* = crossveinless, *lz* = lozenge, *Va* = Venae abnormes.

v w, *car w*, and *v car w* were all phenotypically white but the combination *v car* gave a light yellow eye colour like honey in *D. melanogaster*. As one of the recombinants in each crossover interval will be white due to interaction, the crossing-over percentages are calculated on the non-white half only. From this test the gene order is shown to be

$$y — v — car — sn — w$$

The recombination value of $v — car$ is 31.98 %, of $v — ev$ 40.4 %, of $car — sn$ 17.15 %, of $sn — w$ 32.0 % and of $ev — w$ 38.05 %. Thus the gene order is:

$$y — car — ev — sn — w$$

Together with the 3-point tests the gene order of all the eight mutants must be:

$$sc — y — v — car — ev — sn — N — w$$

In a small experiment the recombination value between $m — w$ is determined as 26 %. As no 3-point test was made with this gene before it was lost, it is not decided whether it lies to the left or to the right of *w*. LÜERS (1938) gave the distance between *sn* and *m* as about 42 units and between w^{co} (an allele to *w*) and *m* as 24 units (fig. 1). Thus it is most probable that *m* lies to the right of *w*. As a sex-linked lethal is found in the $v — w$ crossings to lie at about 20 units to the right of *w*, genes are earlier found on this side of the *w* locus.

TIMOFÉEFF-RESSOVSKY (1931) has constructed a genetical chromosome map of about 85 units (fig. 1 d). A comparison between that map and the one given

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in this paper shows that TIMOFÉEFF-RESSOVSKY has used only mutations from the proximal part of the X-chromosome. If *m* is placed just below *m* from LÜERS, it is seen that *ev* and *sn* will have the same localisation as in the map constructed here. Only *N* is in a different position, if it is really the same mutation in both cases. TIMOFÉEFF-RESSOVSKY has made no 3-point test with *N*. He has made only two crossings to *ev*. If the crossing-over percentages are calculated from all the crossover flies in his experiment and not from the small crossover group only, the percentages will be 24 % and 25 % respectively. Thus according to these experiments *N* could lie 25 units to the right of *ev*. This value corresponds to what has been found in the above experiments. (It gives the distance between *N* — *m* 27 units in the map by TIMOFÉEFF-RESSOVSKY and 28 units in map a of fig. 1.)

In map e of fig. 1 is given 13 sex-linked mutations from the combined results of the recombination experiments by TIMOFÉEFF-RESSOVSKY and LÜERS and the experiments described here. In this map the distance between *sc* and *y* is uncertain.

As it is not proved that *sc* is situated at the extreme left of the chromosome, only the map distances between adjacent mutations are given. The genetical map has no zero-point. The total map distance is 158 units.

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VI. SALIVARY GLAND CHROMOSOMES IN THE PROGENY OF SOME MUTANTS

The *y*, *v*, *sn*, and *w* mutations used in the recombination experiments were shown to have no inversions or other chromosomal aberrations which could decrease the crossing-over frequency. The other mutations have not been investigated cytologically.

The salivary gland chromosomes of all fertile mutants found after irradiation have been tested. In these, a *yellow* mutation was found to be deficient for some bands in 1A4—5 (according to the salivary gland chromosome map of PERJE 1954). A *vermilion* mutation had an inversion in 1D4—1E2 and a reciprocal translocation with chromosome 2, with the breakage points at 1G5a and 2C2a. A *singed* mutation showed a long inversion from 1C1a to 1E5d. In a *white* mutant a deficiency in some bands in 1G3 was found. A *Notch* mutation had a reciprocal translocation between the X-chromosome and chromosome 3, the breakage points being 1G3a and 3B1a. The locations of these chromosomal aberrations within the X-chromosome are shown in fig. 2. From this is seen that the *yellow* end of the chromosome is probably the free end and the *white* end the centromere end. It is not however proved that the aberrations are associated with the mutations. Further cytological investigations are necessary before the exact position of these genes on the X-chromosome is determined.

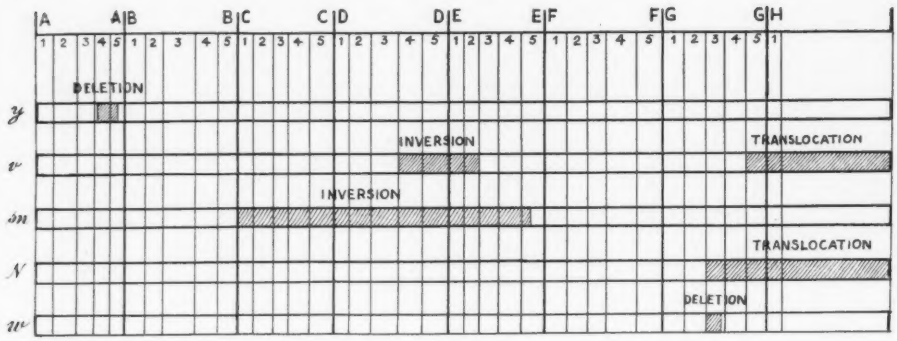

Fig. 2. A diagram showing the position of rearrangements in the X-chromosome of *D. funebris* in a *y*, *v*, *sn*, *N*, and *w* mutation. (The system of band designation as by PERJE 1954).

VII. X-RAY INDUCED MUTATIONS

Since the papers by TIMOFÉEFF-RESSOVSKY on the qualitative and quantitative differences in mutability between *D. melanogaster* and *D. funebris*, it has been regarded as difficult to obtain eye colour and body colour mutations in the latter fly. The question will arise as to whether the mutation frequency in specific gene loci is considerably lower in *D. funebris* than for the corresponding genes in *D. melanogaster*.

It is desirable in an investigation of this kind to work with loci regarded as homologous in the different species, as such genes can be considered identical in origin although functionally they may no longer be so. The difficulties in establishing homology of the loci of two species that do not cross are great, and no evidence can be given that homologous loci are taken in this experiment, where the mutations *y*, *v*, *sn*, and *w* have been used. The location of the *yellow* gene near to a *scute* gene in the free end of X makes it probable that the *yellow* mutations are homologous in the two species. As in *D. melanogaster* and in *D. virilis* the *white* mutation is found in the neighbourhood of *Notch*. STUBBE and VOGT (1940) found evidence for homology between a sex-linked *vermilion* in *D. funebris* and *vermilion* in *D. melanogaster* by transplantation tests. It is not possible to trace homology of *singed* with a particular mutation in *D. melanogaster* but it could correspond to *singed* or *forked* in that species.

The X-ray experiments were carried out as described in the section II. The F_1 offspring were examined for *y*, *v*, *sn*, and *w* mutations and the mutants progeny-tested in order to classify first for fertility and among the fertile progeny for lethality (table VII).

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TABLE V. Number of mutations in marked loci after irradiation with 3240 r of wild type males from the L strain of *D. funebris*.

	F_1 flies observed ♀♀	♂♂	number of mutant females *y*	*v*	*sn*	w^a	*w N*	*N*
number of flies	41 944	40 883	7	9	13	14	3	10
per 10^4			1.7	2.2	3.1	4.1	—	3.1
D. virilis (GIRVIN, 1949) per 10^4 (at 3 047 r)			1.6	2.1	6.7	3.9	—	—
D. melanogaster (LÜNING, 1952) per 10^4 (at about 3 000 r, converted by a factor 3.3 from 960 r)			2.0	—	1.4	2.3	—	—

Table V gives the total number of flies examined, and the mutations found. At the *white* locus 17 mutations were detected. Of these, 4 were higher allelomorphs of *white*, 1 being light red and 3 being yellow. As a comparison the mutation frequencies at a dosage of about 3000 r are given for *D. virilis* and *D. melanogaster*. Even if this experiment is too small to give any reliable values for the mutation frequencies and to show significant differences between the mutation rates, it is quite clear that the mutation frequencies of *D. funebris* are of the same order of magnitude as in *D. virilis* and *D. melanogaster*. Thus there is no cause to assume a different mutability in the individual loci of the eye colour and body colour genes of that fly.

TABLE VI. Number of non-disjunctions, hyperploids and gynandromorphs after irradiation with 3 240 r of wild type males from the L strain of *D. funebris*.

F_1 flies observed ♀♀	♂♂	♀♀ *y v sn w*	♂♂ +	♂♂ *y w*	♂♂ *sn w*	♂ +
41 944	40 883	83	29	1	23	20

The non-disjunction flies found in F_1 were of three types (table VI):

1. *y v sn w*-females, carrying two X-chromosomes from the mother and a Y-chromosome from the father.

2. Wild type males carrying the Y from the mother and the X from the father. These flies ought to be fertile, if the X-chromosome did not contain an irradiation induced sterility mutation. They were the product of secondary non-disjunction.

3. Wild type males of XO type, with the X-chromosome from the father and no sex chromosome from the mother. These are earlier found to be sterile in *D. funebris* by LÜERS (1937). From the 28 wild type males checked, 3 were fertile and 25 sterile (table VII). This suggests that the incidence of non-disjunction is rather high in the *y v sn w*-stock. Probably many of the *yellow*

TABLE VII. The mutants from table V and the non-disjunction males and hyperploid males from table VI tested for sterility. The fertile mutants were checked for lethals in the progeny.

mutant type	total number of mutants	fractional mutants	fertile		sterile	undetermined
			viable	lethal		
y	7	1	3	—	3	1
v	9	—	4	—	5	—
sn	13	2	3	1	9	—
w^a	14	—	6	1	7	—
w N	3	—	—	—	3	—
N	10	—	—	7	3	—
+ ♂ ♂	29	—	3	—	25	1
sn w ♂ ♂	23	—	—	—	16	7

females found in the X-ray experiment described by PERJE (1954) are due to non-disjunction.

Sperms with a deficient X-chromosome may produce hypoploid females or hyperploid males depending on the length of the segment lost. No hypoploid females deficient for more than one of the loci investigated were found, but 24 hyperploid males were detected (table VI). Of these 23 were of *sn w* phenotype. These probably resulted from deletions with one break between *y* and *sn* and the other to the right of *w*. The only *y w* male had up-turned wings and died some hours after hatching. It was probably deficient for the *y* part and the *w* part of the X, but had a fractioned X with the normal *sn*-allele. All the hyperploid males were sterile (table VII), which should be expected if they were of the XO type. None was shown to have intersexual characteristics.

The flies classified as gynandromorphs (table VI) were of three different types. Of the 20 flies, 15 showed *y sn* phenotype on some part of the body, indicating elimination of the paternal X. In the 8 cases where the X-chromosome was eliminated in the tissue of the head, the eyes were partly white. Obviously most or all of these gynandromorphs had the whole paternal X eliminated. 3 other gynandromorphs had the phenotype *w sn* without showing any pattern of yellow. These flies must have been gynandromorphs where only a part of the paternal X had been eliminated. The *yellow* mutation in the maternal chromosome must have been covered by a normal allele from a fragment of the paternal X. As is shown from the hyperploid males in table VI, tissues with one whole X and one fragment of the *yellow* part of the X chromosome gave male characteristics. The third kind of gynandromorphs were those produced by elimination of the maternal X-chromosome. They were more difficult to discover, as they had normal phenotype, without marker genes. 2 such cases were found. No intersexual characters were seen.

The conclusion from these experiments is that the four genes tested (*y v sn*

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and *w*) were similar to the corresponding mutations in *D. virilis,* where a comparable experiment was carried out by GIRVIN (1949). All the viable mutants tested showed total penetrance of the mutant character. Some qualitative differences from *D. melanogaster* could be seen. As many as 4 of the 14 mutations at the *white* locus were higher allelomorphs. Certainly, these experiments are too small to infer that coloured alleles of the white series are more common than in *D. melanogaster* but other experiments point in the same direction. A qualitative difference in the *y* locus is obvious as earlier suggested by PERJE (1954). All the viable *y* mutations obtained had yellow body colour and black bristles rather than yellow bristles which is the most common condition in *D. melanogaster.* Nevertheless it is probable from the position of *yellow* near to *scute* on the left part of the chromosome that these are homologous in the two species. In *D. virilis* different alleles are found at the *y* locus showing different degrees of coloration, *y* with black bristles being not uncommon. The *vermilion* mutations are darker than in *D. melanogaster.* It can be concluded from these results that most of the mutations affecting colour are more deeply pigmented in *D. funebris* and *D. virilis* than in *D. melanogaster,* probably due to the darker and more pigmented wild type of the two former species.

GIRVIN (1949) compared the relative frequencies of viable and lethal mutations of *D. virilis* and *D. melanogaster,* and found the viable mutations to be more common than in *D. melanogaster* with a dosage of about 3000 r. As seen in table VII there is a relatively high frequency of viable mutations in *D. funebris* also.

VIII. DISCUSSION

The X-chromosome of *D. funebris* has two sections, a distal euchromatic and a proximal heterochromatic part. As it is shown by PERJE (1954) that there must be a Y-homologous part in the X-chromosome carrying several genes, it is important to know to what extent the X-chromosome of this species is homologous to the X-element of other Drosophila species.

This investigation has shown that many characteristic genes present in the X-chromosome of other Drosophila species, such as *y, sc, v, sn,* (or *f*), *N, w, lz,* and *bb,* also occur in *D. funebris.* The cytological analysis shows that these genes are distributed throughout nearly the whole length of the euchromatic part of the X-chromosome, indicating that this part is homologous to the X-element of other Drosophila species investigated.

TIMOFÉEFF-RESSOVSKY (1931) has obtained a sex-linked lethal with a homologue in the Y-chromosome giving twice as many males as females. With crossing-over experiments he found it to lie between *Va* and *m.* This will not necessarily mean that a Y-homologous part of the X lies between *Va* and *m* in the

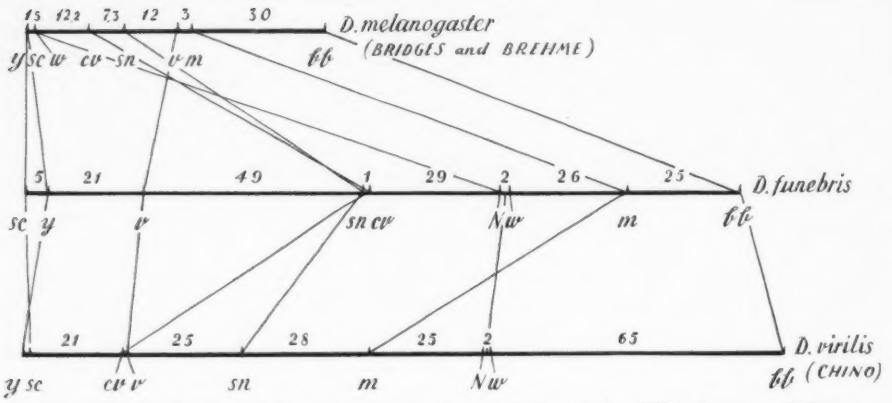
15 122 73 12 3 30
D. melanogaster
(BRIDGES and BREHME)
y sc w cv sn v m bb
5 21 49 1 29 2 26 25
D. funebris
sc y v sn cv N w m bb
21 25 28 25 2 65
D. virilis
bb (CHINO)
y sc cv v sn m N w

Fig. 3. Genetical maps of *D. melanogaster*, *D. funebris* and *D. virilis*, showing differences in the total map lengths and the position of some of the genes found in *D. funebris*.

normal X-chromosome of *D. funebris*, as such a segment could have been inserted in this interval through rearrangements after irradiation. Those experiments were carried out before salivary gland chromosomes were utilized for the analysis of rearrangements.

In fig. 3 a diagram is given for the X-chromosomes of *D. melanogaster*, *D. funebris* and *D. virilis*. The total lengths of the chromosomes are derived from the summation of known map distances between the gene loci. The total length for *D. melanogaster* is 66 crossing over units, compared with 158 units in *D. funebris*. As the latter distance will increase with the location of new genes between those known, it is probable that the genetical maps of *D. funebris* and *D. virilis* will be nearly three times as long as that of *D. melanogaster*. This must depend on the greater amount of crossing over in these two species as compared with *D. melanogaster* as the euchromatic parts of the X-chromosomes are of about the same cytological length in all three species. Some genes that are possibly homologous are plotted on the maps and connected with lines to show their different positions in the chromosomes.

D. funebris is a member of the subgenus Drosophila, but the relationships between this fly and the other members of the subgenus have not been sufficiently investigated. PATTERSON and STONE (1952) showed in fig. 34 a diagram modified after STURTEVANT of the relationships of the genus Drosophila. In this diagram the placing of the funebris group is only indicated by a dotted arrow showing the uncertainty in the relationships. *D. funebris* has many traits common with *D. virilis*. Such similarities as the great amount of crossing-over (fig. 3) are probably characteristic for the whole subgenus Drosophila (PATTERSON and STONE, 1952), but the gene order in the X-chromosome, where *y*, *sc*, *v*, *sn*, *N*, *w*, and *bb* (fig. 3) of the 14 investigated mutants have about the same position in the X-chromosome of both species suggest a closer relationship

than that given by PATTERSON and STONE. On the other hand gene orders in *D. funebris* and *D. hydei* (another member of the subgenus Drosophila in the repleta group) are quite different (PATTERSON and STONE, 1952).

It is obvious from this investigation that the differences in the X-chromosomes of *D. funebris* and *D. virilis* are mainly due to the length of the proximal heterochromatic segment. The euchromatic part only differs in intrachromosomal rearrangements, causing alterations in the linear arrangement of whole blocks of genes.

IX. ACKNOWLEDGEMENTS

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I am greatly indebted to my teacher Professor G. Bonnier for his guidance and encouragement during the work and to Mrs. Anita Alm for valuable technical assistance. I also wish to offer my thanks to Miss M. E. MacKendrick for revising the English text.

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X. SUMMARY

1. Viability tests are made for 6 sex-linked mutations, of which only *ev* showed higher viability than the wild type.

2. Recombination values are given for 9 different sex-linked genes in the non-homologous part of the X-chromosome.

3. The gene orders and map distances are constructed for 14 sex-linked mutations, five of which are taken from the data of TIMOFÉEFF-RESSOVSKY (1931) and LÜERS (1938).

4. Cytological investigations of the salivary gland chromosomes of some of the mutations indicate that *y* is situated at the free end of the chromosome and *w* at the centromere end.

5. X-ray induced mutations in the *y*, *v*, *sn*, and *w* loci suggest that the mutation frequencies of these loci are of the same order of magnitude as in *D. melanogaster* and *D. virilis*.

6. The X-chromosome of *D. funebris* is compared with that of *D. virilis* and that of *D. melanogaster*. It is seen that *D. virilis* and *D. funebris* have nearly three times the amount of crossing-over found in *D. melanogaster*, in spite of the same type of sex-linked genes being distributed throughout the whole chromosomes.

Some gene groups such as *y* — *sc* and *w* — *N* are found in all three chromosomes. The position of *bb* at the centromere end is common to all three species.

An analysis of the distribution of some known mutations shows that most of the X-chromosome of *D. funebris* must be of the same origin as that of *D. virilis*. Intrachromosomal rearrangements of the gene material can be the cause of the differences in the euchromatic part of the two chromosomes.

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VARIATION IN THE MANIFESTATION OF THE SCUTE MUTATION IN DIFFERENT STRAINS OF DROSOPHILA FUNEBRIS

BY

ANN-MARGRET PERJE

(Institute of Genetics, University of Stockholm, Stockholm, Sweden)

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I. INTRODUCTION

A sex-linked mutation affecting the scutellar bristles was found in a Swedish population of *Drosophila funebris* (PERJE, 1954). Because of its phenotypical similarity to the *scute* gene in *D. melanogaster* it was named *scute*.

The phenotypic effects of a number of different *scute* mutations of *D. melanogaster* have been carefully studied by SEREBROVSKY et alii (1930) which showed that in a large series of *scute* alleles every *scute* mutation gave distinctly different patterns of bristle reduction. Particular bristles are absent in each case, but there is always a certain amount of variation. From these facts SEREBROVSKY (1930), and DUBININ and FRIESEN (1932) in the Russian school developed a hypothesis of step arrangement of the alleles.

STURTEVANT and SCHULTZ (1931) discussed the step allelomorphism in the *scute* complex. Although they changed a few details of the step order, they agreed mainly with the step allele hypothesis and they concluded that the normal allelomorph of the *scute* gene is not a single unit but that several subgenes are each concerned with the development of one or a few bristles on the fly.

CHILD (1936), however, doubts that it is a pattern problem in the *scute*

series. He has made temperature experiments with different *scute* alleles and he has found that each bristle reacts independently of all the other bristles. He criticises the hypothesis of bristle pattern in the following way: "If the mean bristle numbers and not the individual flies are considered, the impression one obtains is that certain bristles are affected more than others, in a definite pattern. When the individuals are considered separately, however, it is seen that there is no consistent pattern. The distribution of bristles on one fly may differ in any way from that of another fly." In no cases has he found significant association coefficients between bristles. GOLDSCHMIDT (1938) does not consider CHILD's arguments to be valid.

NEEL (1940) has obtained the same results as CIHLD (1936) with some genes causing extra bristles, while PLUNKETT (1926) has found in *Dichaete* a definite pattern of bristle distribution with association coefficients of 1 (total association) between bristles close to each other.

SPARROW and REED (1940) in an investigation of the *extra bristle* complex in *D. melanogaster* and its reaction with *scute*[8] showed significant associations between certain bristle pairs, and assumed that there must be a determination stream which originates at the anterior scutellar bristles and diffuses in all directions but more rapidly anteriorly.

On the other hand, MULLER (1932) has concluded by studying gynandromorphs with different *scute* alleles that there must be an autonomous development of each bristle.

The bristle reducing mutation *scute* in *D. funebris* shows a very distinct pattern of distribution, where in most cases the anteriorly scutellar bristles (asc) are absent but the post scutellar bristles (psc) present. The thoracic bristles are not affected. The variations in these conditions are investigated in a great number of flies.

The continuous variation in bristle number in the expression of *scute* in *D. melanogaster* is known to be sensitive to differences in modifying genes (HASKELL, 1943 and COCKS, 1954) and to environmental factors (PLUNKETT, 1926 and IVES, 1939). Thus it would be desirable to study the *scute* effect in isogenic stocks and at constant temperature. In *D. funebris* no crossing-over inhibitors are known for the different chromosomes, so that it is not as easy to construct isogenic stocks as in *D. melanogaster*. Instead of avoiding modifying genes, this investigation has been carried out by crossing the *scute* mutation to eleven different wild type stocks which were certain to have different modifying genes, in order to decide to what extent the modifiers can change the penetrance and expressivity of the *scute* gene.

The present paper is mainly concerned with the following problems:

1. Does the *scute* gene in *D. funebris* affect the same bristles in all the individual flies and is there any association in the *scute* effect on the asc with psc bristles?

2. Are the variations of the *scute* effect of the same kind in homozygous *scute* females as in hemizygous *scute* males?
3. To what extent is the *scute* gene affected by genetical modifiers, if introduced into other wild type stocks?
4. Is the penetrance of the *scute* effect in heterozygous females changed by modifiers?

II. MATERIAL AND METHODS

scute is a spontaneous, sex-linked mutation from the L strain (PERJE, 1954), and the *scute* stock was established from a *scute* male crossed to a wild type L female, after which a heterozygous daughter was backcrossed to the *scute* father. The *scute* progeny was bred in small mass cultures, and selection was made for modifiers for about 25 generations, only flies with no anterior scutellars but with both post scutellars being chosen. Even after this selection period there was always a variation in the phenotypes. The bristle effects produced were completely absence and not reduction in size.

The Drosophila breeding methods as described by PERJE (1954) were used. As it is known from *D. melanogaster* (CHILD, 1935), that the effect on the bristles can be modified by environmental factors such as temperature, the experiments were carried out in as uniform an environment as possible in a constant temperature room at 25°C.

In the following experiments the number of scutellar bristles has been counted on every fly. Only in cases where no circular socket remained was the bristle classified as missing.

Three different kinds of experiments were carried out. At first the bristles on the scutellum in *scute* flies from pair matings were counted. In the second experiment about 2000 flies in mass cultures from each wild type strain were classified for scutellar bristle number. In the third experiment crosses were made between the *scute* stock and eleven different wild type strains (S) in both types of cross; *sc*♀ × S♂ and S♀ × *sc*♂. Twenty pair matings were made in each case and the scutellar bristles were counted in the progeny. The strains employed were the same as described by PERJE (1954).

III. THE MANIFESTATION OF *SCUTE* IN THE *SCUTE* STOCK

The *scute* mutation of *D. funebris* has many characteristics similar to the *scute* mutations of *D. melanogaster*, as it is sex-linked and its locus in the X-chromosome is situated near to *yellow*. But unlike most of the *scute* mutations of *D. melanogaster*, it usually reduces the anterior scutellar bristles only.

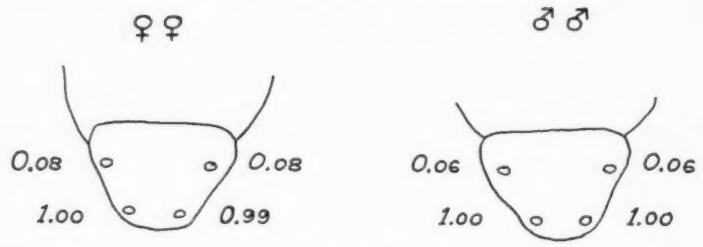

Fig. 1. The frequency of scutellar bristles in females and males in the *scute* stock of *Drosophila funebris*.

Hypomorphic mutant genes in *D. funebris* usually do not give differences from the normal phenotype as marked as the corresponding mutants in *D. melanogaster*. Examples of this are the *scute* mutation, the *yellow* mutations which in most cases have yellow body colour but black bristles, and many bristle and wing mutations with low penetrance and expressivity.

This *scute* mutation has a very distinct pattern of distribution. The bristle distribution is determined from the progeny of pair matings in the *scute* stock. Fig. 1 gives the frequency of each scutellar bristle from 523 females and 404 males. The variation in the *scute* effect is shown in table I and fig. 1. Some of the flies have 4 scutellar bristles and thus demonstrate non-penetrance of the *scute* gene. The flies with 4 bristles have been crossed and their progeny showed the *scute* character to the same extent as the flies with *scute* phenotype. The penetrance is higher than 99 % (table I).

TABLE I. The scutellar bristle number, penetrance and mean in females and males of *scute* in *Drosophila funebris*.

Stock	Number of scutellar bristles				Penetrance of *scute*	Mean bristle number
	4 (= 2 asc + 2 psc)	3 (= 1 asc + 2 psc)	2 (= 2 psc)	1 (= 1 psc)		
sc ♀♀	4	75	442	2	99.24 ± 0.39	2.16
sc ♂♂	2	46	355	1	99.51 ± 0.35	2.12
Totals	6	121	797	3	99.35 ± 0.27	2.14

In the flies with 3 bristles, it is always one of the asc bristles which is reduced. The remaining asc bristle is as long as in wild type flies, but sometimes it is bent inwards or placed nearer to the psc bristle of the same side than in the wild type flies. In all cases observed with only 2 bristles, it was the asc bristles that were absent (in 13 485 flies). In no case where a psc bristle was missing were the asc bristles present. This indicates a very high degree of association between asc and psc bristles. PLUNKETT (1926), CHILD (1935), and SPARROW and REED (1940) have given the association coefficients between

bristles on the same side in *D. melanogaster* mutations. While CHILD (1935) did not find any significant association between the bristles from different *scute* mutations, PLUNKETT in work on *Dichaete* and SPARROW and REED on *extra bristles* found significant association between some bristles situated near each other. The formula for the association coefficient given by SPARROW and REED (1940) is not valid if less than about 1 % of the bristles of one kind are missing. As this is the case with the psc bristles in the pure *scute* stock which has been selected for the two anterior scutellar bristles in 25 generations, no association coefficient could be determined there. It is only stated that no exception is found to the rule that if a psc bristle is lacking, both the asc bristles are absent. In the experiments with *sc* ♀ × S ♂, reported below (table III), the variation in the *scute* effect of the males is greater, as crossing to the wild type has broken up the modifying systems selected for in the *scute* stock. A significant association coefficient of 1 is calculated from the pooled values of the *scute* males from these crossings. The total association between the asc and psc bristles is consistent with the pattern hypothesis (SPARROW and REED, 1940) which says that there is a maximum effect on a particular bristle area for each mutation and where the expressivity is high, there can be a spread of the effect to adjacent bristles. As in the *extra bristle* mutations described by those authors, the center of the *scute* effect investigated here is situated at the asc bristles, but unlike *extra bristle* it spreads posteriorly.

In some bristle mutations earlier described (ROKIZKY, 1931) in *D. melanogaster* differences in manifestation are found between the two sexes. As shown in table I the bristle mean is slightly lower and the penetrance is somewhat higher in males than females. These differences are not significant, indicating that the homozygous females have the same manifestation of the character as the hemizygous males.

IV. VARIATION IN THE SCUTELLAR BRISTLE NUMBER IN THE DIFFERENT WILD TYPE STRAINS

Eleven different strains, the same as described by PERJE (1954), have been used in the cross experiments below. The variation of the scutellar bristles were determined from data on 2000 flies from small mass cultures from each strain (table II). χ_c^2-tests (χ^2 with Yates' correction for continuity) gave no significant differences between males and females *within* the strains, but significant differences *between* some of the strains when each strain was χ_c^2-tested against the pooled values of all the other strains (table II). It is known from *D. melanogaster* (CHILD, 1935) that temperature differences during larval and pupal stages and other environmental factors in the laboratory cultures give no variation in bristle number in wild type flies, which usually have built up

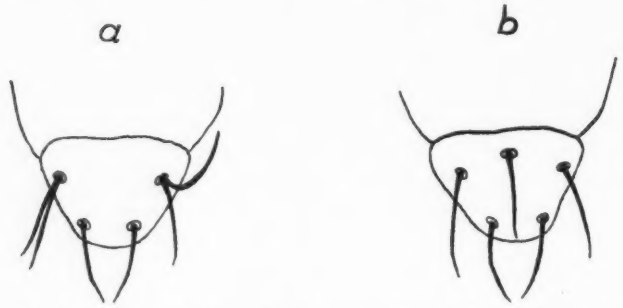

Fig. 2. *Extra bristles* in the +A, I, and W strains (fig. 2a) and in the K strain (fig. 2b) of *Drosophila funebris*.

TABLE II. The scutellar bristle number in females and males of different wild type strains of *Drosophila funebris*. Each strain is χ_c^2-tested against the pooled values of all the other strains.

Strain	Number of scutellar bristles ♀♀				♂♂			χ_c^2	Probability
	6	5	4	3	5	4	3		
+A	1	4	1024		5	1036		11.26	< 0.001
+B			949	28		1011	21	144.67	< 0.001
B			864	2		1031	2	3.54	0.06 —0.07
E			935	3		1041	4	1.06	0.30 —0.50
H			965	4		1048	8	0.03	0.80 —0.90
I		1	1143			1002	1	9.77	0.001—0.002
K			1044	1		1025	5	2.18	0.10 —0.20
L			1025	2		1018	4	2.08	0.10 —0.20
M			863			1022		10.10	0.001—0.002
P			948	17		997	15	44.92	< 0.001
W			1076	2		1048	1	6.16	0.01 —0.02

a gene content that acts as a buffer against environmental influences. Some of the differences in these experiments could be due to more or less highly developed buffering systems in the different strains, but as the environmental conditions are relatively constant, probably most of the variation depends on differences in the polygenic systems that work with genes of small, similar and supplementary effect on the increase or decrease in the number of bristles.

A particular phenotypic manifestation is the increase in bristle number, earlier found in several different species of Drosophila, as in *D. melanogaster* (Sparrow and Reed, 1940) and *D. virilis* (Girvin, 1949), and described by Spencer (DIS 1) in *D. funebris* as the mutant *extra*. This mutation gives extra bristles close to the asc bristles on one or both sides of the scutellum. *Extra bristles* of this kind are found in both +A and I and in the crosses with W (table III and fig. 2a). Another kind of *extra bristle* is found in the

crosses with the K strain (table III) where the extra bristle is situated in the middle of the scutellum between the two asc bristles (fig. 2 b). In no case has it been possible to make pure stocks with these *extra bristle* genes. In the other strains and crosses no extra scutellar bristles have been found.

In all these strains no flies are found with less than 3 bristles. As 2 bristles is the most common manifestation of the *scute* gene used in this investigation, the strains are obviously free from that mutation.

TABLE III. The scutellar bristle number in females and males from the crossing S × *sc* and *sc* × S in *Drosophila funebris*. χ_c^2-tests of the bristle number of females from reciprocal crosses are made to investigate homogeneity. Flies from the wild type strains are χ_c^2-tested to the males from the corresponding strains in the crossing S × *sc*, and males from *sc* × S are compared to the pure *scute* stock.

Strain	Number of bristles ♀♀ from S × *sc*				Number of bristles ♀♀ from *sc* × S					χ_c^2	Probability
	5	4	3	2	5	4	3	2	1		
+ A	2	1322	7			298	3	3		4.89	0.02—0.03
+ B		1120	401	50		402	158	18	2	0.71	0.30—0.50
B		2364	75	1		724	25			0.03	0.80—0.90
E		4099	344	7		1718	201	16		18.01	< 0.001
H		7470	1198	85		3507	778	65	1	47.69	< 0.001
I		1406	33			957	72	5		36.40	< 0.001
K	2	1485	257	51		410	70	3		1.00	0.30—0.50
L		692	53	3		749	49	1		0.73	0.30—0.50
M		3158	120	1		2403	87	5		0.00	> 0.98
P		727	85	2		1165	142	18	1	0.90	0.30—0.50
W	3	1481	70	1	1	860	30			1.77	0.10—0.20
Totals	7	25324	2643	201	1	13193	1615	134	4		

Strain	Number of bristles ♂♂ from S × *sc*				χ_c^2	Probability	Number of bristles ♂♂ from *sc* × S					χ_c^2	Probability
	5	4	3	2			4	3	2	1	0		
+ A	45	1301	2		(40.25)	< 0.001	3	54	268	5	3	2.03	0.10—0.20
+ B		1387	63	1	9.75	0.001—0.002	1	62	539	5	2	3.51	0.06—0.07
B		2338	4	1	0.00	> 0.98	25	201	504	1		71.27	< 0.001
E		4258	80		21.21	< 0.001	3	211	1692	9	1	3.56	0.06—0.07
H		8560	48	9	0.04	0.80—0.90	7	280	3985	30	3	50.86	< 0.001
I	3	1346	3		0.96	0.30—0.50	37	275	757	1		68.31	< 0.001
K	2	1858	43		30.08	< 0.001	14	103	395	5		18.22	< 0.001
L		760	6		2.11	0.10—0.20	26	147	653	3	3	15.10	< 0.001
M		3105	4		1.09	0.20—0.30	35	503	1989	12		24.09	< 0.001
P		698	14		0.20	0.60—0.70	51	308	924	8	3	61.49	< 0.001
W	3	1556	6		1.30	0.20—0.30	36	218	635	2	1	58.85	< 0.001
Totals	53	27167	273	11			238	2362	12342	81	16		

V. THE CROSS EXPERIMENTS

In order to investigate differences in variation and penetrance of the *scute* phenotype in the different wild type strains (= S) the following two types of crosses were carried out: wild type females from the eleven strains mentioned above were crossed to *scute* males (S × *sc*) in twenty pair matings for each strain, and *scute* females were crossed to males from the different wild type strains (*sc* × S) in twenty pair matings. A check was made that all wild type flies employed in the crosses had four scutellar bristles and all the *scute* flies two bristles (the two psc bristles). The results are given in table III. As the difference between the pair cultures within a series was of no importance (as seen in table VI), the sum of the progeny of the twenty pairs is given in the table.

A. WILD TYPE MALES FROM THE CROSSES S♀ × *sc*♂

These males are of the normal phenotype in most cases, as the X-chromosome comes from the wild type line and the Y-chromosome from the *scute* line. The agreement of χ_c^2-test on those hybrid males and the flies from corresponding wild type strain (table II) is shown in table III. As the column with 5 bristles has been added to the 4-bristle column and the one with 2 bristles to the 3-bristle column, the χ^2 is given for one degree of freedom. Only in three cases (+B, E, and K) is there significance in the deviation of bristle number from the corresponding wild type strain. If the 5-bristle column is treated per se in the χ_c^2-test in the +A strain there is significant deviation in that case also (the χ_c^2 within the parenthesis in table III).

In fig. 3 the average bristle numbers of the wild type males in the strains and those of the hybrid males from the crossing (S × *sc*) are compared. It may be said that 4 bristles is the normal number in wild type, even if plus and minus variation may occur in the cultures. This number is represented by the horizontal line in fig. 3. It is evident that in the only strain with plus variation (+ A), the average of the bristles numbers has increased in the hybrids, but in the strains with minus variation the means have further decreased in such cases where there have been any significant changes in bristle number. The most probable explanation of this condition seems to be that mutants showing low penetrance and affecting bristle number are suppressed by a well established genic system of modifiers within the strain. If a disturbance of the equilibrium between main genes and modifiers is called forth by crossing with a strain which has built up another bristle gene modifying system, the penetrance of the mutant character will increase to some degree. Probably the plus and minus variation depends on different genic systems.

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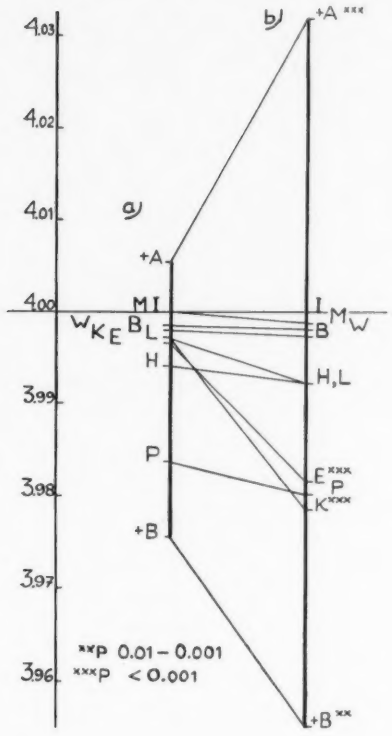

Fig. 3. The means of scutellar bristles in males of the different strains of *Drosophila funebris* (fig. 3 a) compared to those obtained by outcrossing the strains to *scute* males (fig. 3 b).

B. HETEROZYGOUS FEMALES

The females from these two crosses ought to have the same genotype. χ_c^2-tests for the variation between females of reciprocal crosses show good agreement in most cases, only in the +A, E, H, and I strains are there obvious significant differences (table III). The cause of these differences is difficult to determine as reciprocal crosses between the particular strain and *scute* are raised simultaneously with the parents taken from the same mass cultures for both crosses. In order to estimate whether the variation is high among the

TABLE IV. χ_c^2-tests for the first and second ten pair cultures *within* the experiments in the crossings with strain E, H, and I of *Drosophila funebris* that showed highly significant differences between females from reciprocal crosses (table III).

Strain	*sc* × S		S × *sc*	
	χ_c^2	Probability	χ_c^2	Probability
E	3.43	0.06—0.07	1.22	0.20—0.30
H	23.54	< 0.001	11.45	< 0.001
I	0.01	0.90—0.95	1.39	0.20—0.30

twenty pair matings of any particular experiment a χ_c^2-test is carried out for the pair cultures where the first ten cultures are compared with the remaining ten within the same experiment. As seen from table IV the agreement between the first and second half of the experiment is good for E and I, but in the H strain there is a significant heterogeneity within the experiments. As in both E, I, H and +A the mean is lower in the crosses *sc* × S than in the reciprocals (table V), it is possibly some maternal effect from the *scute* females that decreases the bristle number.

TABLE V. The mean scutellar bristle number and percentages of flies with less than four scutellar bristles in the crossing S × *sc* and *sc* × S in *Drosophila funebris*. The correlation coefficients are given between the percentages of less than 4 bristles of S males and S/*sc* females (r_1), between S males and *sc*/S females (r_2), and between S males and *sc* males (r_3).

Strain	Mean				Penetrance percent			
	S × *sc*		*sc* × S		S × *sc*		*sc* × S	
	♂♂	♀♀	♀♀	♂♂	♂♂	♀♀	♀♀	♂♂
					(x)	(y_1)	(y_2)	(y_3)
+A	4.032	3.996	3.970	2.147	0.15	0.53	1.97	99.10
+B	3.955	3.681	3.655	2.090	4.41	28.71	30.69	99.84
B	3.997	3.968	3.967	2.342	0.21	3.11	3.34	96.58
E	3.982	3.920	3.880	2.108	1.84	7.89	11.21	99.84
H	3.992	3.844	3.791	2.060	0.66	14.66	19.40	99.84
I	4.000	3.977	3.921	2.325	0.22	2.29	7.45	96.54
K	3.979	3.801	3.843	2.244	2.26	17.16	15.11	97.29
L	3.992	3.921	3.936	2.228	0.78	7.49	6.26	96.88
M	3.999	3.963	3.961	2.221	0.13	3.69	3.69	98.62
P	3.980	3.891	3.864	2.306	1.97	10.69	12.14	96.06
W	3.998	3.956	3.968	2.321	0.38	4.57	3.37	95.96
				Correlation coefficients		$r_1 = 0.909$	$r_2 = 0.866$	$r_3 = 0.329$
				Probability		< 0.001	< 0.001	0.30—0.40

The dominance relations between a gene and its normal allele are known to be affected by changes in other parts of the genic system. This is shown among others for some *scute* alleles (STURTEVANT and SCHULTZ, 1931) and *vestigial* (GREEN and OLIVER, 1940) in *D. melanogaster* and for *Scute* in *D. pseudoobscura* (HELFER, 1939). In table V the percentages of flies showing decrease in scutellar bristles are given for the two crosses in table III. For the heterozygous females and the hemizygous males these values correspond to the penetrance of the *scute* effect. It is seen in the heterozygous females that the *scute* mutation can show partial dominance from 0.5 % to about 30.7 % when combined with different strains. The correspondence between the reciprocal crosses is relatively good. The correlation between the percentages of bristles in the males (x) from the S × *sc* crosses and the females (y_1) of the same cross is determined. The correlation coefficient (table V) is $r_1 = 0.909$

and is highly significant. This indicates that the gene system acting on scutellar bristle formation in wild type flies is of great importance in influencing the dominance of the *scute* gene in the heterozygous females. The males and females compared in this correlation coefficient are from the same cross. If there are environmental disturbances conditioned by the breeding methods in these experiments they will mainly be of the same kind *within* a vial for both females and males but be of different kinds *between* the different vials. Thus environmental differences should increase the correlation between the males and females. In order to avoid these kinds of environmental influences, the correlation coefficient is also determined for the same males but with the females from the reciprocal crossing. Also in this case there is a high correlation, the correlation coefficient being $r_2 = 0.866$.

That the variation in the dominance obtained in heterozygous *scute* females are not only the additive effect of a constant penetrance frequency of the *scute* mutation and the effect on the same bristles by other genes in the wild type strains is shown by an analysis of variance (table VI) carried out as follows: the first fifteen pair matings from each cross experiment were arranged in

TABLE VI. Analysis of variance of the scutellar bristle mean in the crosses S × *sc* (VI a) and *sc* × S (VI b).

VI. a. S × *sc*

Source of variation	Degrees of freedom	Sum of squares	Mean squares
Between strains	10	0.320424	0.032042***
Between sexes	1	0.256606	0.256606***
Between groups	4	0.008143	0.002036
Interaction: strain—sex	10	0.172061	0.017206***
Residuals	84	0.162896	0.001939
Total	109	0.920130	

VI. b. *sc* × S

Source of variation	Degrees of freedom	Sum of squares	Mean squares
Between strains	10	1.109072	0.110907***
Between sexes	1	75.193582	75.193582***
Between groups	4	0.025428	0.006357
Interaction: strain—sex	10	0.183915	0.018392**
Residuals	84	0.454700	0.005413
Total	109	76.966697	

** Probability 0.01—0.001
*** Probability < 0.001

five groups with three pairs in each, taken in order. In every group the scutellar bristle mean was determined. The variation of these means between strains, between sexes (which correspond in the first analysis to wild type males and $+/sc$ females, and in the second analysis to $sc/+$ females and sc males) and between groups, and the interaction between strain and sex is shown in table VI. The variance between groups is not significantly greater than the residual variance, thus the groups within a strain can be regarded as samples from the same population. Significant differences are found between the different strains and between the sexes that represent different genotypes. The interaction between the strain and sex in table VIa is highly significant. Genetically, this indicates that the genic systems working in the wild type to form the scutellar bristles give a greater effect in the heterozygous *scute* females than should be expected on the basis of simple summation of a constant partial dominance effect of *scute* and of the effect of independently working bristle genes from the wild type strains. Such effects could be a consequence of intra-allelic gene interaction (dominance), caused by different wild type alleles of *scute* in the strains, or of inter-allelic gene interaction (epistasis, complementary effects, and so on) caused by non-specific modifying systems that alter the *scute* effect. As shown from table VIb in the variance analysis of the reciprocal crosses, there is also significance (P: 0.01—0.001) in the interaction between strain and sex ($sc/+$ females and sc males). This cannot be due to intra-allelic effects as the males are hemizygous for *scute*, but must depend on modifiers that can be of either non-specific type or of specific type, acting only on the penetrance of the *scute* mutation. The interaction is not necessarily caused by the same phenomenon in the reciprocal crosses. It is not possible in this investigation to decide whether the interaction between wild type males and heterozygous *scute* females is due only to inter-allelic effects or to a combination of intra- and inter-allelic effects, but obviously some of the variation must depend on autosomal modifying genes.

C. *SCUTE* MALES FROM THE CROSSES $sc♀ \times S♂$

Although the same scute allele is used in all cases, its combination with the different wild type strains will give no two of the eleven strains having the same phenotypic expression, as shown by the variation of scutellar bristles in the *scute* males from the different strains (table III). χ_c^2-tests, with the frequencies of bristle numbers in the pure *scute* stock taken as the expected values show significant differences in the bristle number for eight of the eleven crosses. In all cases the mean bristle number is about 2 (table V), indicating that in spite of influences by other genes most of the flies show the typical *scute* effect with only two psc bristles.

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VI. CONCLUSIONS

The scutellar bristle number in *D. funebris* lends itself to exact quantitative estimation as it is composed of discrete units which can be counted with practically no errors in observation.

The variability is due partly to the environment and partly to modifying genes. In the experiments accounted for in this paper, the only measures taken against environmental differences are breeding in a constant temperature room and using pair cultures or small mass cultures in order to avoid overcrowding. Variations due to environmental conditions ought to be more or less random. The results of the crossings here indicate that the environmental influences are small in comparison with the variations depending upon modifying genes (table VI).

PLUNKETT (1926) has given the following definition of modifying genes: "Genetic modifying factors for bristle number are genes which show no effect on bristle number by themselves, but only when a bristle reducing gene is also present." In this paper, the word modifiers is used in a wider sense. Even if the genes give a phenotypic manifestation in the wild type strains, they are discussed as modifiers to the *scute* gene, which is regarded as the main gene in this investigation. The *extra bristle* gene is earlier described as a major gene (SPENCER, DIS 1) but with less than 5 % penetrance. Probably the observed effect is the resultant of many slight interactions between the genes. It is a matter of convenience to make a distinction between main genes and polygenes, the mutation under investigation being considered as the major gene and all other genes affecting its phenotypic manifestation as belonging to its modifying system. In *D. funebris*, where most of the mutants affecting wing size, wing shape, wing margin, bristles, legs, among other characters show low penetrance and much variable expression, it is often impossible to distinguish between the main gene and its modifiers.

The situation found in these experiments may be explained thus. The wild type strains are small populations heterogeneous for polygenic combinations, balanced against each other by selection, so that the result will be a phenotypic uniformity with only a few exceptional flies. If the polygenic combinations in different populations are balanced in different ways and no interbreeding has taken place between the strains, the balance is disturbed in the hybrids. This can explain the increased variability both in the wild type males and the heterozygous *scute* females in the cross experiments.

Dominance has been considered by FISHER (1928, 1929) to be established by modifying systems and by HALDANE (1930, 1933) and S. WRIGHT (1929, 1934) by different wild type allelomorphs. In this case it is clear that much of the variability in the dominance of the *scute* gene must depend on the same

modifying factors that cause the phenotypic effect in the wild type males, as there is a high correlation between the two phenomena. Whether there are also different wild type allelomorphs of the *scute* gene in the strains cannot be determined from these experiments.

As no correlation is found between the penetrance of the bristle effects in the wild type strains and in the *scute* males from the crosses *sc* × S (table V), some of the genes are probably specific modifiers, working only in combination with the *scute* gene. Other modifiers, however, have been shown to be operative in the same way in the mutant and non-mutant groups, thus being non-specific modifiers affecting the manifestation of the character in the presence of both wild type and mutant allelomorphs.

VII. ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Prof. Gert Bonnier for his encouragement and criticism during the course of the investigation. For technical assistance I am much indebted to Mrs. Anita Alm and for revising the English text to Miss M. E. MacKendrick. The work was carried out with aid of a grant from the Swedish Natural Science Research Council.

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VIII. SUMMARY

1. The mutation *scute* in *D. funebris* is introduced into 11 different wild type strains (= S). The scutellar bristles are counted in the progeny, in order to investigate the variation in the manifestation of the *scute* phenotype.

2. This *scute* mutant has a very distinct pattern of distribution with an association coefficient of 1 between the asc and psc bristles. In no case is a psc bristle missing without a coincident absence of both asc bristles.

3. There is no significant difference between the *scute* effect in hemizygous males and homozygous females in the *scute* stock.

4. The scutellar bristles are counted in about 2000 flies of each wild type strain. No differences are found between males and females *within* a strain but significant differences are found *between* the strains. Some of the strains show *extra bristle* effect.

5. In the males from the crosses S × *sc* the variability in the number of scutellar bristles is significantly greater than in the corresponding wild type strain in four cases. This possibly depends on the wild type strains being heterogeneous for polygenic combinations that are balanced by selection, so that they give few exceptional flies, and on the balance being disturbed in the hybrids.

6. The *sc*/+ and +/*sc* females from the crosses in most cases show homogeneity. In only a few strains is the number of bristles significantly lower in the progeny of the crossing with *scute* females than in the reciprocals, possibly depending on a maternal effect from the *scute* mother.

7. The heterozygous females show partial dominance varying from a few percent in some strains to about 30 percent in others. That the manifestation of the dominance is partly due to interaction with bristle modifying genes in wild type strains is shown by an analysis of variance.

8. The *scute* males from the crosses *sc* × S show significant differences in eight of the eleven cases when χ_c^2-tested against the pure *scute* stock. This indicates that modifiers from the different wild type strains change the manifestation of the *scute* effect.

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GENETICAL ANALYSIS OF A STRAIN WITH AN EXCESS OF MALES IN DROSOPHILA FUNEBRIS

BY

ANN-MARGRET PERJE

(Institute of Genetics, University of Stockholm, Stockholm, Sweden)

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I. INTRODUCTION

Radiation-induced sex-linked recessive lethals in the Y-homologous part of the X-chromosome have been obtained by TIMOFÉEFF-RESSOVSKY (1931) and PERJE (1954) in *D. funebris*. These lethals give twice as many males as females (sex ratio = 2/3), if females heterozygous for the lethal are crossed to heterozygous males.

In natural populations of the same species, more males than females often occur (PERJE, 1954). By determining the sex ratios after pair matings in such populations it is shown that only a few of the pair cultures have a sex ratio of about 1/2 and of about 2/3 which ought to be the most common values, if lethals of the kind described above occurred. Most of the sex ratios are distributed between these two values. This condition indicates that a more complicated genic system is in action. MOHR and STURTEVANT (1919) have described the same phenomenon in a strain of *D. funebris* from Norway, but have interpreted the variation between the number of males and females as depending on environmental conditions. In this paper, selection experiments have been carried out for some generations in order to increase the number of males in the cultures.

II. MATERIAL AND METHODS

The strains employed are W from Witwatersrand, South Africa, representing a line with a normal frequency distribution of the sex ratios and M from Melbourne, Australia, which was the strain with the highest sex ratio mean in the experiments accounted for by PERJE (1954). Furthermore, a synthesized mutant stock with the mutants *yellow, vermilion, singed,* and *white,* has been used to find out if the same phenomenon occurred in a stock constructed of mutants from three different strains (E, L, and P) all of which were found to have high sex ratios.

The selection experiments were carried out as follows: from a pair culture with a relatively high sex ratio (determined as the quotient of all males to all flies) about a hundred brother-sister pair cultures were raised and their progeny counted. From one of these cultures with a high sex ratio, about a hundred new pair matings were carried on for a further generation and their sex ratio determined. The same process was repeated for four generations.

Selection experiments of this kind present some difficulties. First the sex ratio cannot be determined until all flies in the culture have emerged. Thus the flies must be taken from a culture which in the first days of counting has more males than females. However, after a further few days the sex ratio may have stabilized (PERJE, 1954), and is not as high as expected. Secondly, if a culture has many more males than females, too few pair cultures can be achieved for the next generation. Thirdly, as for each generation more than 20 000 flies must be counted, only a few selection lines can be followed at the same time.

The breeding methods have been the same as described by PERJE (1954).

III. RESULTS

A. THE W STRAIN

In the W strain 90 brother-sister matings were made from a pair culture with the sex ratio 0.501. As is the case in the following experiments, all cultures with less than 50 flies were rejected in order to avoid uncertain sex ratios. The frequency distribution of the sex ratios is given in the histogram of fig. 1 a. The results are reported in table I. The sex ratio determined from all flies emerging in this generation was 0.502. Obviously this distribution of the sex ratios could be expected if no lethals occur in the crosses.

In fig. 1 a a frequency curve, calculated from the normal distribution with the harmonic mean of the number of flies per culture taken as the actual number of individuals, is superimposed upon the histogram. As seen from

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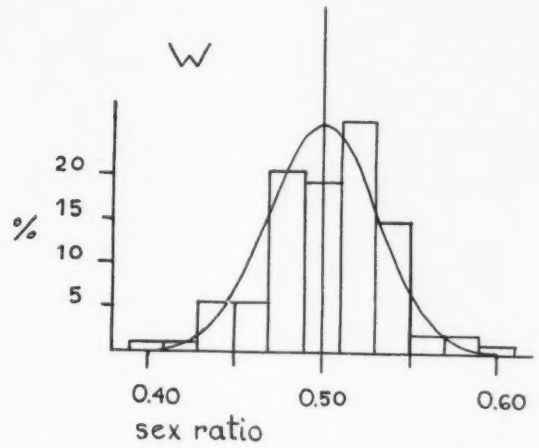

Fig. 1 a. Histogram showing the frequency distribution of pair cultures with different sex ratios in brother-sister pair matings from a parent culture with the sex ratio of 0.501 in the W strain of *D. funebris*. A normal curve is superimposed upon the histogram.

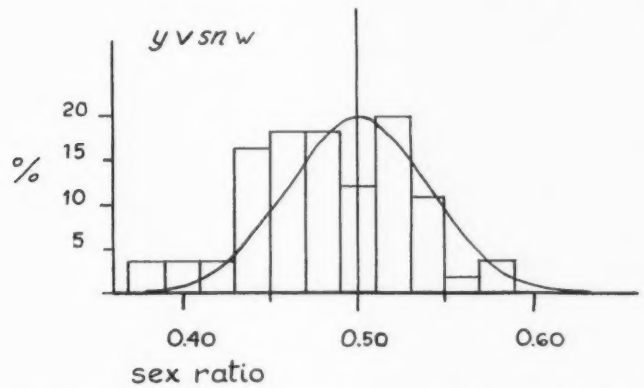

Fig. 1 b. Histogram with superimposed normal curve of the frequency distribution of pair cultures with different sex ratios in a *y v sn w* stock of *D. funebris*.

this, the histogram representing the sample and the continuous frequency curve representing an estimate of the form of the hypothetical population show relatively good agreement.

B. THE *y v sn w* STOCK

This mutant stock is constructed of the mutant *yellow* from the P strain, *vermilion* from the E strain, *singed* from a mutant stock received from Norway (not investigated for the distributions of sex ratios), and *white* from the L

strain. As seen in fig. 9 (PERJE, 1954) all the strains from which the mutants *y*, *v*, and *w* originated, had a distribution of the sex ratios with the frequency of 0.35 to the left and 0.65 to the right of the 0.5-line.

73 brother-sister matings were made from a culture with the sex ratio 0.498. The frequency distribution of the sex ratios of 62 cultures are given in the histogram in fig. 1 b and the data of the experiment in table I. In spite of the parental strains having a rather high sex ratio, the *y v sn w* stock showed no such tendencies. Moreover, there were more females than males, the total sex ratio being 0.480. This low sex ratio is expected if no lethals occur in the stock, as all the mutants are sex-linked and have slightly lower viability in hemizygous males than in homozygous females.

C. SELECTION EXPERIMENTS WITH THE M STRAIN

Two series of selection experiments (MA and MB) were carried out, MA beginning with a pair culture with the sex ratio 0.533 and MB with 0.548. In four generations selection for high sex ratio was continued, each experiment originating from one pair culture. The Roman numerals in table I mark the generation in question, the small letters indicate that several experiments are made simultaneously within the same generation. In table I the number of flies from each experiment is given and the sex ratios are calculated from these values. It is seen that there is an obvious increase in the sex ratio of each generation, even if it is relatively low in the parent culture as in MA IV a and MA IV b.

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TABLE I. Sex ratio experiments in *D. funebris*. The sex ratios in the original cultures, the number of flies from the pooled values of females and males from all pair cultures in each experiment and the total sex ratios calculated from these values.

Experiment	Sex ratio in the parent culture	Number of flies ♀♀	♂♂	Total	Number of cultures	Sex ratio
W	0.501	13 691	13 799	27 490	90	0.502
y v sn w	0.498	6 122	5 661	11 783	73	0.480
MA I	0.533	37 490	42 204	79 694	198	0.530
MA II	0.563	23 020	26 565	49 585	136	0.536
MA III	0.585	18 025	22 806	40 831	132	0.559
MA IV a	0.515	19 774	25 311	45 085	144	0.561
MA IV b	0.498	22 011	29 103	51 114	191	0.569
MB I	0.548	26 315	29 899	56 214	134	0.532
MB II	0.570	18 673	21 750	40 423	117	0.538
MB III a	0.568	11 316	14 478	25 794	81	0.561
MB III b	0.812	4 877	7 689	12 566	48	0.612
MB IV b	0.876	9 645	17 121	26 766	92	0.640

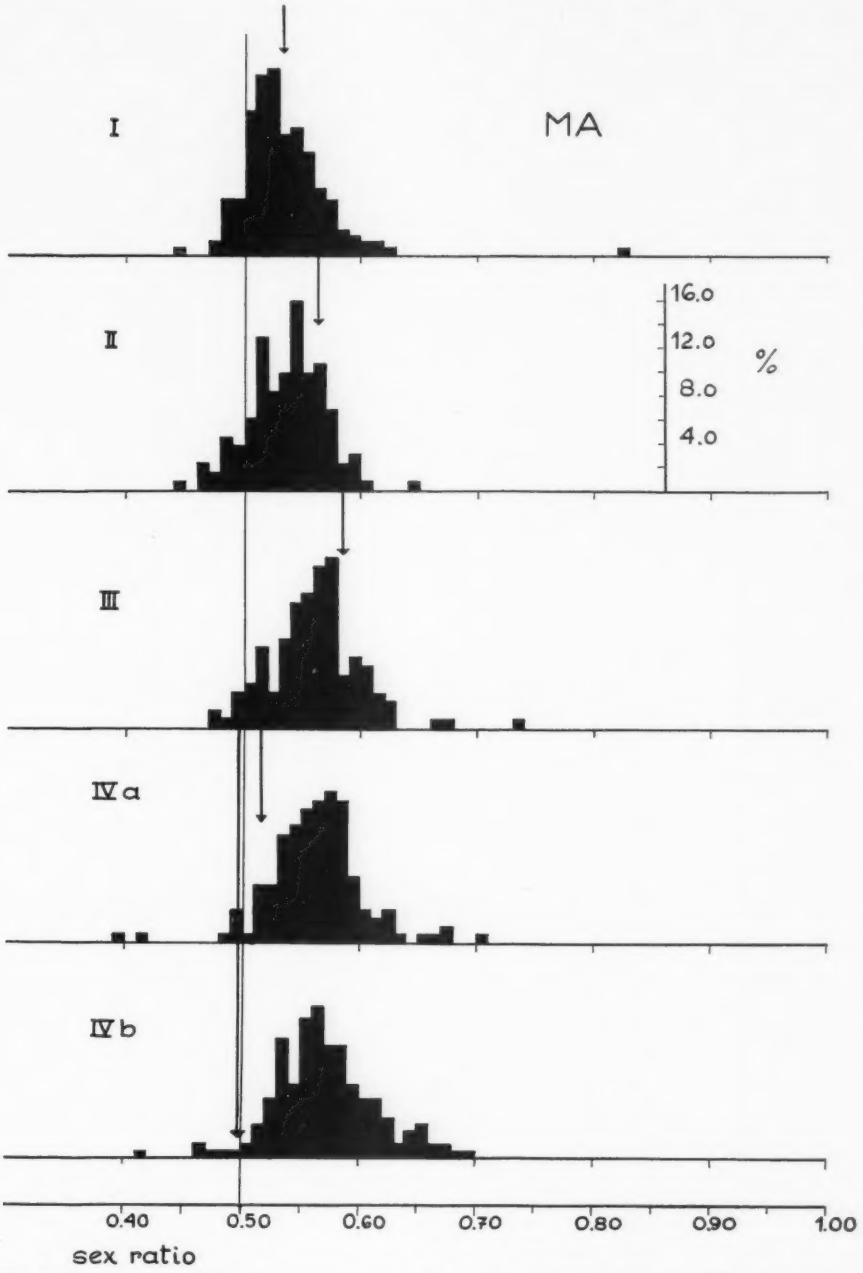

Fig. 2. Histograms of the frequency distributions in four generations (I—IV) after selection for an excess of males in the selection lines MA of the M♀ strain of *D. funebris*. The arrows indicate the sex ratio of the parent culture from which the pair cultures are made.

TABLE II. The frequency distribution of pair cultures with different sex ratios in the W strain, the *y v sn w* stock, and the two selection experiments MA and MB in *D. funebris*. The sex ratio means for the pair cultures in each experiment is given below.

Sex ratio	Frequency of pair cultures											
	W	*y v sn w*	MA					MB				
			I	II	III	IV a	IV b	I	II	III a	III b	IV b
0.37—		2										
0.38—												
0.39—						1						
0.40—	1	2										
0.41—	1					1	1					
0.42—		2										
0.43—	1	3										1
0.44—	4	6	1	1					1			2
0.45—	2	6						1				1
0.46—	3	4		3			2	2	1	1		
0.47—	12	1	2	2	2		1	1	1	1		1
0.48—	6	9	9	6	1	1	1	3	3			2
0.49—	5	5	9	5	4	4	1	13	5			5
0.50—	12	2	24	8	5	1	2	8	12	4	1	5
0.51—	12	6	30	17	9	7	5	16	10	4		7
0.52—	10	5	31	11	4	7	9	22	18	6	3	6
0.53—	5	3	20	13	10	13	18	10	9	4	4	1
0.54—	8	3	21	21	14	14	11	14	14	10	4	4
0.55—	1	1	17	13	15	16	21	12	14	9		3
0.56—	1		11	14	18	17	23	9	17	9	2	1
0.57—	2	1	9	9	19	18	17	12	2	6	1	
0.58—			4	3	6	17	17	5	2	9	1	1
0.59—	1	1	3	4	8	8	11	2	2	8	1	
0.60—			2	1	7	4	9		3	6	3	1
0.61—			2		4	3	9			2	3	
0.62—			1		3	4	6	1		2	4	1
0.63—						1	2		1		3	4
0.64—				1			4				2	1
0.65—						1	5		1		1	
0.66—					1	1	2				3	1
0.67—					1	2	2				4	2
0.68—							1				1	1

Table II shows the frequency distribution of the sex ratios in the different experiments. If the sex ratio means are compared to the sex ratios in table I which were calculated from the pooled values of all the males and females within an experiment, it is seen that for high sex ratios the means of the frequency distribution are slightly higher. This is a result of the lower number of flies in each culture with a high sex ratio, as would be expected if lethals were in action.

Obviously a relatively high percentage of the cultures rejected for having

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Sex ratio	W	*y v sn w*	Frequency of pair cultures MA I	MA II	MA III	MA IV a	MA IV b	MB I	MB II	MB III a	MB III b	MB IV b
0.69—							1				1	
0.70—						1						1
0.71—												
0.72—												1
0.73—					1							
0.74—												
0.75—												1
0.76—												
0.77—												1
0.78—												
0.79—												1
0.80—												
0.81—									1			
0.82—			1									1
0.83—												1
0.84—											1	2
0.85—											1	2
0.86—												3
0.87—											2	6
0.88—												2
0.89—												1
0.90—												4
0.91—												4
0.92—												3
0.93—											1	1
0.94—												
0.95—												
0.96—												1
0.97—												1
Total	87	62	197	132	132	142	181	131	117	81	47	88
Mean	0.503	0.483	0.533	0.536	0.559	0.563	0.571	0.533	0.540	0.561	0.633	0.680

less than 50 flies, have high sex ratios that are genetically determined. This cannot be avoided as it is necessary to have this degree of confidence in the sex ratios. 17 cultures from MA and 8 from MB were rejected.

The histograms in fig. 2 and 3 give the frequency distributions of the sex ratios of the selection experiments calculated as percentages of pair cultures in each interval. Nearly all the cultures lie to the right of the 0.5-line for sex ratio which is the vertical line in both figures. The arrows show the sex ratios of the parent culture from which the experiments have started. No rapid selec-

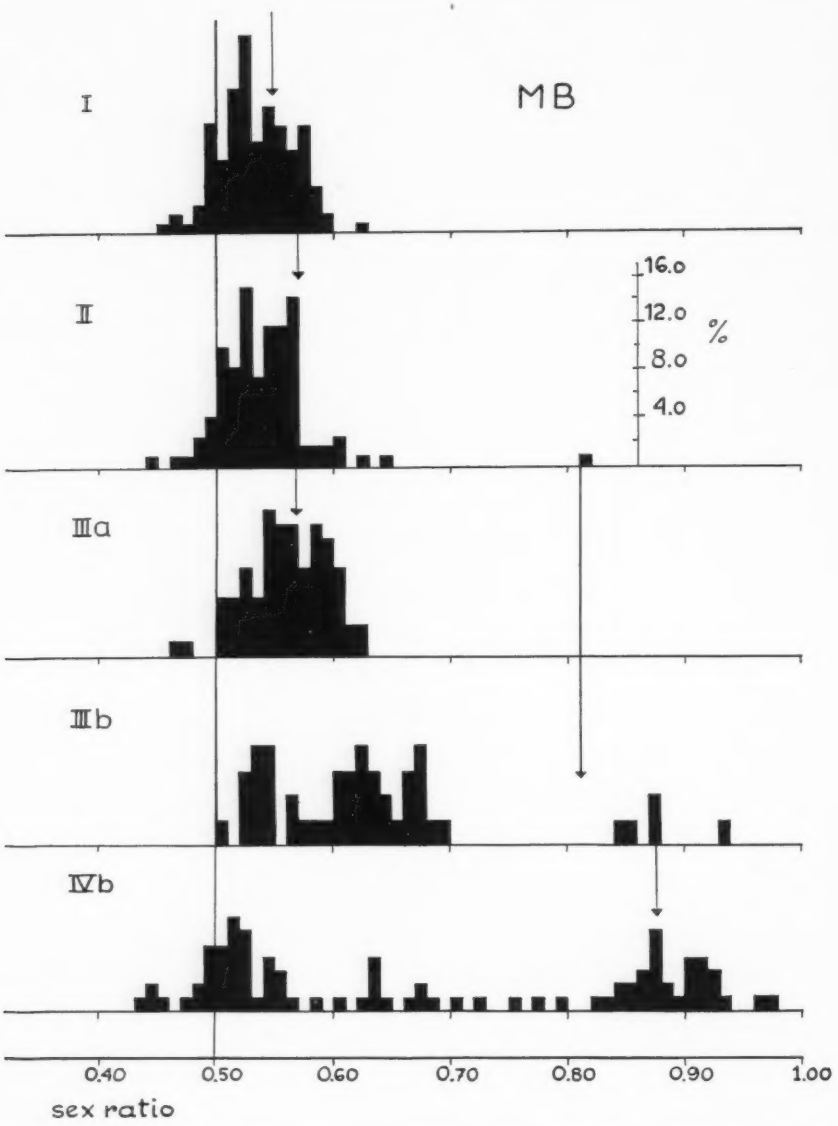

Fig. 3. Histograms of the frequency distribution in the selection lines MB of *D. funebris*.

tion was made, and only a slight shift to the right is seen for each generation. Such a condition must depend on the united action of several balanced lethals. In the experiment MB II one pair culture totally isolated from all the others in the distribution had the sex ratio 0.812. This culture was examined for two further generations (MB III b and MB IV b) where the sex ratios have split

up into many different maxima in MB III b and the whole distribution is spread out showing a new obvious maximum at about 0.86—0.88 in MB IV b.

Selection was carried out in the following four series:

1. MA I — MA IV a
2. MA I — MA IV b
3. MB I — MB III a
4. MB I — MB IV b

As seen from the analysis of variance in table III it can be confirmed that there are great differences between the generation means, P being less than 0.001 in all four series.

TABLE III. Analysis of variance of generation means in the selection experiment MA and MB.

Series	Source of variation	Degrees of freedom	Mean square	Probability
MA I—MA IV a	Between generations	3	0.037553	< 0.001
	Within a generation	599	0.001393	
	Total	602		
MA I—MA IV b	Between generations	3	0.060903	< 0.001
	Within a generation	638	0.001462	
	Total	641		
MB I—MB III a	Between generations	2	0.018619	< 0.001
	Within a generation	326	0.001279	
	Total	328		
MB I—MB IV b	Between generations	3	0.493651	< 0.001
	Within a generation	379	0.009113	
	Total	382		

IV. DISCUSSION

Two different kinds of mutation can give a ratio of 1♀ : 2♂. First there can be a sex-limited autosomal mutation which is a recessive lethal manifesting itself only in females. Secondly, there can be a sex-linked mutation, situated in the Y-homologous part of the X-chromosome. If brother-sister matings are made from the 1 : 2 cultures, there will be different results from these two types of mutations. From the autosomal mutation, half of the pairs will give females to males in the ratio 3 : 4 (sex ratio = 0.57) and the other half of the cultures in the ratio 1 : 2. From the sex-linked mutations half of the cultures will give the ratio 1♀ : 1♂ and the other half 1♀ : 2 ♂.

The X-ray induced lethals from the P strain (PERJE, 1954) were proved to be of the second type with half of the cultures showing a sex ratio of 0.67 and the other half about 0.50. This type of lethal is rare in *D. melanogaster*, but has been described several times (THOMPSON, 1920, BONNIER, 1923, and SCHUBEL, 1934). Furthermore, some lethal *bobbed* mutations have been reported (STERN and OGURA, 1931).

In some of the wild type strains, the sex ratio situation is more complicated (PERJE, 1954). Most of the pair cultures have sex ratios within the range 0.50—0.67, but only a few show the 0.67-value. If pair matings are made from 0.67-cultures there will be no distinct maxima in the frequency distribution of the sex ratios at 0.50 and 0.67 and again only some of the cultures show the 0.67-value. The whole range from 0.50 to 0.67 will arise. Thus it has not been possible to produce a 1 : 2 stock of the kind obtained after X-raying, showing that in nature a more complicated system of balanced lethals must have developed.

This lethal action can be caused by different combinations of recessive lethal mutations. It may be due to a united action of several autosomal lethals or to different sex-linked mutations in the Y-homologous part of the X, or to the combined action of autosomal and sex-linked genes. It is impossible to determine the total number of lethals working in the natural populations, but the selection experiments described above, partly indicate the location of the lethals on the chromosomes. As it was not possible to mark the Y-homologous part of the X or neighbouring parts of it with visible mutants, and no autosomal markers were available, the standard method of measuring the distance of a lethal from known loci could not be used.

If the phenomenon depends on a combination of autosomal genes alone, there must have arisen specific lethal mutations manifesting themselves only in females. On the other hand, if it deals with lethals in the Y-homologous part of the X, or if autosomal recessive lethals have the corresponding normal alleles translocated to the Y-chromosome as a duplicate segment, there is no need for the mutant action to be restricted to females. In such cases the sex ratio in the progeny will depend on segregation, and not on a particular physiological action of the lethals in question. Thus it is more probable that the balanced genic system that has manifested itself in nature is due either to sex-linked genes alone or to a combination of sex-linked (either in the X or in the Y) and autosomal factors rather than on autosomal lethals exclusively.

In the following discussion, the influences on the sex ratios by some lethal systems are discussed in connection with the results obtained in the selection experiments.

As it is known from the radiation experiments that sex-linked recessive lethals can be induced in the Y-homologous part of the X-chromosome (TIMOFÉEFF-RESSOVSKY, 1931 and PERJE, 1954) the first hypothesis suggested

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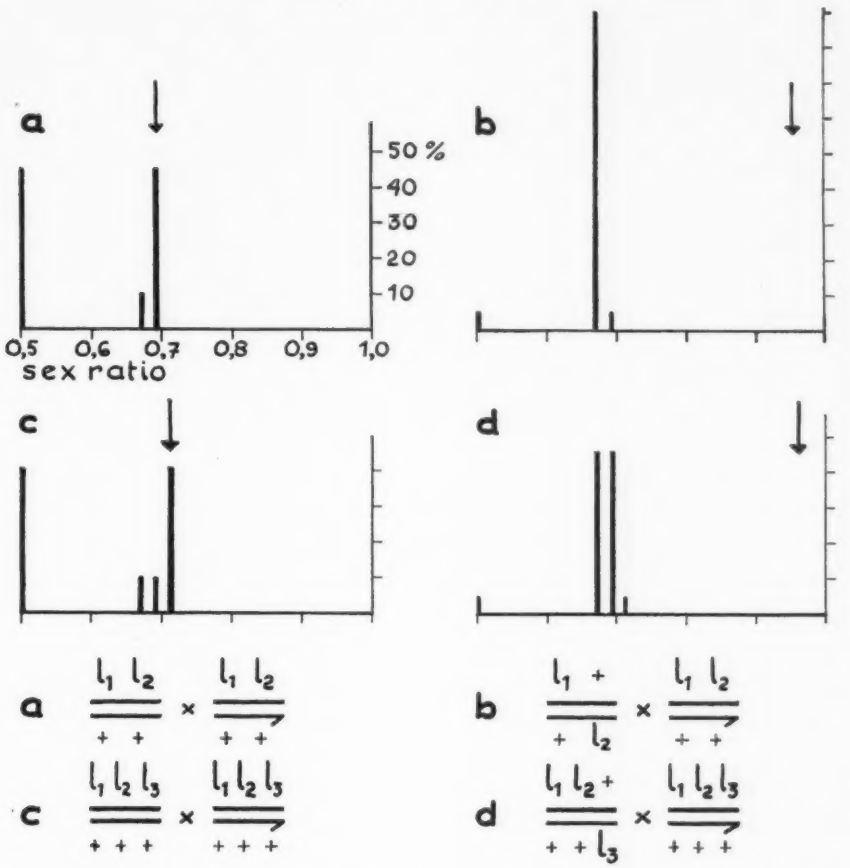

Fig. 4. Frequency polygon of the theoretical sex ratios in some crosses with sex-linked lethals in the Y-homologous part of the X-chromosome. The recombination percentages are assumed to be 10 % between l_1 and l_2 and 10 % between l_2 and l_3. The genotypes of the parents are given below. The arrows show the sex ratio in the parent cultures.

was that a number of sex-linked lethals had accumulated in the part of X which was hidden by normal alleles in a section of Y. As LÜERS (1938) and KIIL (DIS 20) have found dominant mutants in the Y, namely *Bobbed* and *Stub*, it is seen that mutations do occur in the Y-chromosome where a small euchromatic part has also been detected in the salivary gland chromosomes (PERJE, 1954).

In fig. 4 some examples of segregation in sex ratios are shown, when two or three sex-linked gene pairs with normal alleles in the Y-chromosome take part in the action. The arrows point out the sex ratio in the parent culture and the cross refers to the genotypes of the parents. It is obvious that it is

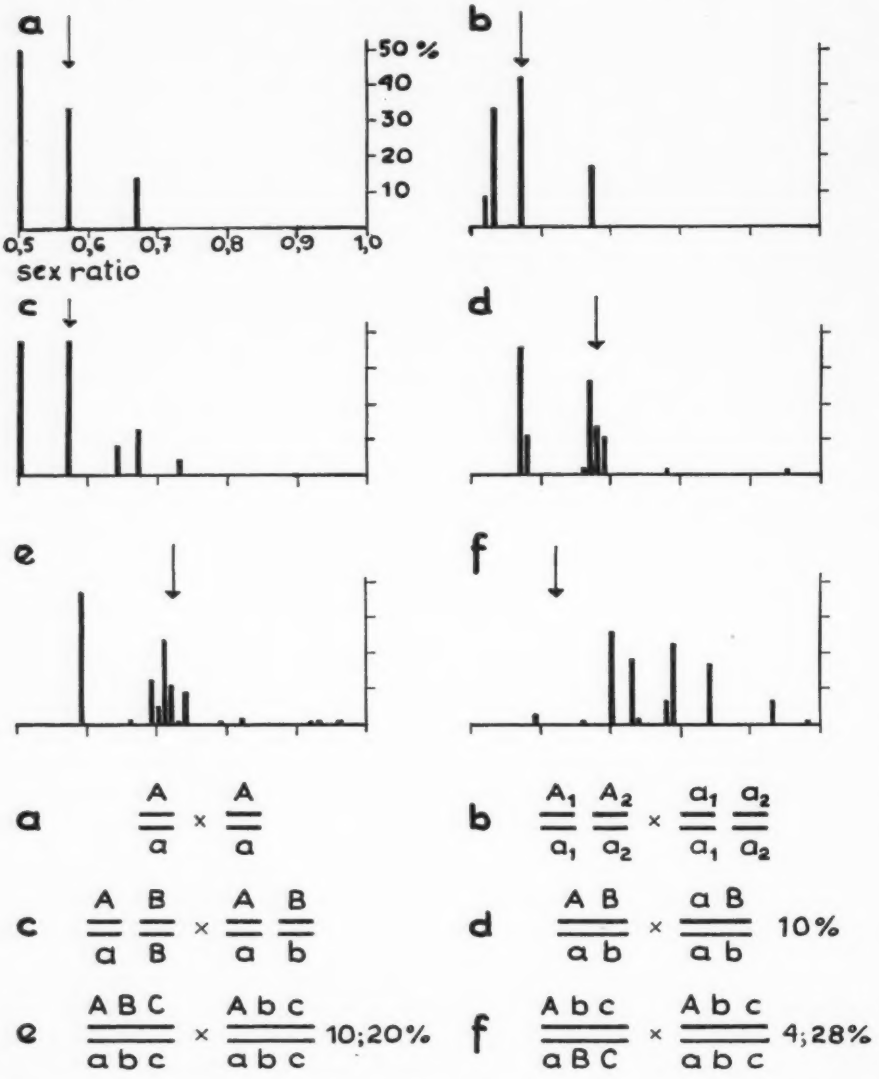

Fig. 5. Frequency polygon of the theoretical sex ratios in some crosses where recessive autosomal lethals are assumed, *b* duplicate factors in different chromosomes, *c*—*f* complementary factors.
The recombination percentages are given after the parental genotypes.

not possible to obtain a frequency maximum *between* 0.50 and 0.67, if only different sex-linked lethals cause the changes. As those sex ratios are most frequent in the selection experiments another mechanism must be in operation.

The second hypothesis made as a basis for the reasoning was that autosomal

recessive lethals gave a sex-limited effect, so that only females died. This effect could be due either to a particular physiological action in the females or as discussed above to a duplicate segment of the chromosome translocated to the Y-chromosome.

In fig. 5 diagrams are shown for some sex ratio distributions with different autosomal combinations. In the case with one gene pair 25 % of the cultures lie to the left of the 0.5-line, which is not the case in the histograms. If two autosomal genes are at work there could be many different types of interaction between them. The two cases discussed here are duplicate effects, corresponding to what should be expected if the same gene is duplicated in another part of the chromosome set, and there should be four recessives of the same kind to obtain this effect; and complementary effects, which correspond to the effect of two independently working lethals. The genes could be linked or located in different chromosomes. Both types of examples are found in fig. 5. The duplicate factors give results in fig. 5 b which correspond strongly with the distribution found in the first generations of the selection experiments. On the other hand two duplicate genes cannot be the only cause of the differences in sex ratios, as it is impossible to obtain sex ratios higher than 0.67 with this gene mechanism, or with such factors situated in the same chromosome either in coupling or repulsion.

If the cases with complementary effects with two or three different lethals are regarded (fig. 5 c—f), it is found that such systems can explain combinations with high sex ratios if it is assumed that the genes are linked. In the cases where the values are high for the sex ratios, it is impossible to obtain at the same time values lower than 0.59. Hence the segregation found in MB IV b in fig. 3 must have another explanation.

Surveying these hypothesis it can be said that to obtain a maximum value of the frequencies at a point between 0.50 and 0.67 it is necessary to have autosomal factors, which most probably operate as duplicates. To obtain values between 0.75 and 1.00 the most probable mechanism would be a complementary effect between linked factors. To explain the segregation in MB IV b when the two maxima are situated at about 0.50 and about 0.86 it is necessary to assume sex-linked genes in the Y-homologous part of the X.

Such a condition could be established if a duplicated part of the Y-homologous section of the X has been inserted in an autosome, or in another part of the X. If this duplication occurs in the homozygous condition, lethals could as easily be spread through the populations as in polyploids. If there is a sex chromosome—autosome system at work and the same gene has mutated to a lethal in both the X and the autosomes, these genes will behave as duplicate factors (fig. 5 b). If the lethals in the X become homozygous but the corresponding lethals in the autosome are still heterozygous, the effect will be as though an autosomal sex-limited lethal is operating. If on the other hand the

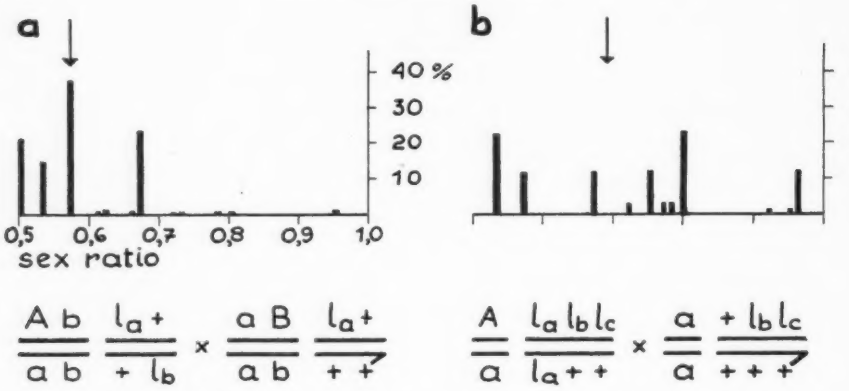

Fig. 6. Frequency polygon of the theoretical sex ratios of a combined autosomal—sex-linked system. A, a—l_a and B, b—l_b act as duplicate factors and A, a—B, b and l_a—l_b as different lethals with 10 % recombination respectively.

lethal in the autosome is the first to become homozygous, the lethal in the X-chromosome will behave as a sex-linked gene with a normal allele in the Y. If rather a long chromosomal section is involved, many different lethals could occur independently of each other. They will give a linked complementary effect as demonstrated in fig. 5 d—f. Depending on which will be homozygous at first they can manifest themselves as sex-linked systems or linked autosomal systems in the course of evolution. This hypothesis can explain why the genes sometimes act as autosomal lethals with sex-limited effect and sometimes as sex-linked lethals. A similar effect can be achieved if a part of the Y-homologous segment of the X is duplicated and inserted in the differential part. If only a section of an autosome has been translocated to the Y-chromosome as in *D. buscki* (KRIVSHENKO, 1952) without the X-chromosome having any corresponding lethals it would not be possible to obtain a segregation of the kind shown in MB IV b with maxima at 0.50 and 0.86.

In fig. 6 some genetic mechanisms are given that begin with parent cultures with rather high sex ratios and that are spread out through the whole range from 0.50 to nearly 1.00. These are combined sex-linked and autosomal systems. Many other autosomal—sex-linked and exclusively sex-linked genic combinations are conceivable to explain the results in the selected lines, and fig. 6 demonstrates only a few possible explanations. It is obvious that in the selection lines MA and MB I—III a (figs. 2 and 3) the gene mechanism acts mainly as an autosomal system (cfr fig. 5 b), and that in the selection line MB I—MB IV b it is the segregation of the sex-linked genes that is observable, but the effect must be modified by autosomal genes.

MATHER (DARLINGTON and MATHER, 1950) has proposed that polygenic systems are restricted to the heterochromatic parts of the chromosomes. This has

been strongly criticised by SIMPSON (1953) among others. In the polygenic system of lethals in *D. funebris* it is obvious that the sex-linked lethals must lie in the heterochromatic part of the X or in the very faintly stained euchromatic bands in the region G 5 to H 1 (PERJE, 1954) of the salivary glands where the X- and the Y-chromosomes pair, and where the bands are indistinct and mostly composed of intercalary heterochromatin. Thus this is another occasion where some of the genes in a polygenic system are located in more or less heterochromatic parts of a chromosome and where linkage is necessary to explain the mechanism.

It is shown in cytological investigations of the salivary gland chromosomes (HANNAH, 1951) that duplicated segments are often more or less heterochromatic. This could possibly depend on the fact that lethals and detrimentals can accumulate in these sections without being selected against in nature, as they are covered by the action of their normal alleles in the other part of the duplicate segment. The reduced efficiency of the genes in a duplication decrease the pairing tendencies in these regions of the chromosomes so that the euchromatic bands will be less distinct.

If in the duplicate segment one of the homologous gene pairs has preserved its normal function and the other gene pair has more or less lost its activity, a system with a major gene and its modifier can develop. In cases where both the genes have mutated to detrimentals they may act as modifiers to other genetic systems. All intergrades between major genes and polygenes may be expected. As tandem duplications are frequently found in the genus Drosophila such modifying systems could be linked and lie in heterochromatic regions in the chromosomes, in a manner such as the polygenes described by DARLINGTON and MATHER (1950). All types of lethal in the segment will work in the same polygenic system, and they must be closely linked to each other within two groups corresponding to the duplicated segments, or if these lie near each other in the same chromosome, all factors will be linked.

The lethal systems common in the strains of *D. funebris* (PERJE, 1954) and giving abnormal sex ratios must have an effect on the evolution of the species. From the analysis of the *y v sn w* stock which is built up from strains containing lethals, it is seen that probably most of the lethals that have accumulated in the strains are non-homologous, as they do not appear in the first generations of the crosses (fig. 1 b). Only after relatively prolonged inbreeding do they manifest themselves again. In nature such lethals will cause many other adjacent genes to retain their heterozygous condition, as their homozygotes are selected against due to linkage with the lethals. Thus, much of the variability must be hidden in the form of balanced combinations of polygenic systems. This could give a possible explanation of the accumulation of modifiers that is so characteristic for the species (PERJE, 1954 and 1955). Inbreeding in the

strains will fix the balanced systems so that the polygenic mechanism can combine uniformity within the species with a high degree of heritable variation. As recombinations occur in the females, combinations of more extreme effect are occasionally produced. If artificial selection is imposed on such recombinants the balanced system can be broken up as has been the case with MB III b and MB IV b. It may be expected that such a pool of available variation within a species, will make it capable of adjustment to differences in environment. If new fixed polygenic systems are again built up the evolution of the species as a whole may be slow.

It is usually taken for granted that the Y-chromosome has evolved from a chromosome with a normal complement of genes, originally homologous with those of the X-chromosome. During the course of development the Y-chromosome has lost most of its genes and usually differs morphologically from the X. If during this development lethal mutations occur in Y-homologous parts of the X-chromosome they will establish themselves in the population more easily than those in the Y-differential part of the X, which are always subject to a very strong negative selection pressure. If such lethals are widespread in the population, natural selection will prevent lethal genes and deficiencies from remaining in the corresponding parts of the Y. Thus in the course of evolution these parts of the Y may become stabilized.

In such organisms where the Y-chromosome has been found to carry genes, it is very possible that lethal systems have developed in the X, so that a part of the original Y-chromosome has stabilized. In such cases, the Y-homologous part of the X has many opportunities to accumulate lethals, as these will be distributed in the population in the same ways as autosomal recessive mutations. If the ordinary XY-mechanism determines sex in such a species it is probable that populations with more males than females will be found.

In man all the pre-requisites for such a system occur. It is known that Y-limited factors occur, such as those for *porcupine* and *webbed toe* (STERN, 1949) and that there is crossing over between the X and Y (STERN, 1949) indicating that the two chromosomes are partly homologous. This evidence implies that a relatively active Y-chromosome is functioning. In such a situation, X-chromosome lethals may accumulate in the populations. It is well established that more males than females are born in man and that the sex ratios are different in different populations. One possible genetical explanation of this phenomenon is that sex-linked lethals have accumulated in the Y-homologous part of the X in the populations. Furthermore, these lethals may be modified by a more complicated polygenic system as is the case in *D. funebris*.

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VI. SUMMARY

1. Brother-sister matings were made from a pair culture in the W strain of *D. funebris* with the sex ratio 0.502. There was a normal frequency distribution of sex ratios, showing no sex-linked lethals in the system.

2. Brother-sister matings from a pair culture of the *y v sn w* stock of *D. funebris* with the sex ratio 0.498 gave no deviations from the normal sex ratio distribution other than should be expected from the lower viability of the mutant hemizygous males. The sex ratios in the original strains from which the mutant stock was built up were abnormal.

3. Selection for high sex ratios was made in four lines of the M strain of *D. funebris*. The generation mean increased significantly. Only slight differences could be found between the generations. In the series MB III b and MB IV b the balanced polygenic system was broken up and showed a spread of sex ratios from about 0.5 to nearly 1.0 with two obvious maxima in MB IV b, one at about 0.52 and the other at about 0.86—0.88.

4. The frequency distribution in MB IV b (fig. 3) shows that sex-linked lethals in the Y-homologous part of the X must be a part of the system. Such sex-linked lethals cannot be the only cause of the abnormal distribution, as with such a system no theoretical sex ratio values should be expected between 0.50 and 0.67. Thus there must be an interaction between the sex-linked lethals in the Y-homologous part of the X and lethals in some other part of the chromosome set. As the mechanism appears partly to be due to duplicate factors and partly to linked complementary gene actions the author has proposed the hypothesis that duplicated segments may be the cause of this phenomenon. One of the segments should lie in the X- and the Y-chromosomes and the other in one of the autosomes, or possibly be inserted in the non-homologous part of X. Homologous gene pairs would act as duplicate factors and different lethals within a segment as linked complementary factors.

5. Such a balanced system of lethals will cause many adjacent recessive mutations to retain their heterozygous condition, which will give a gene mechanism that combines uniformity of the species with a high degree of heritable variation. This will give a possible explanation of the accumulation of modifiers which is a characteristic of the species.

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ANALYSIS OF SYNAPSIS IN SALIVARY CHROMOSOMES OF LARVAE FROM DIFFERENT STRAINS OF DROSOPHILA FUNEBRIS

BY

ANN-MARGRET PERJE

(Institute of Genetics, University of Stockholm, Stockholm, Sweden)

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I. INTRODUCTION

In a previous paper (PERJE, 1954) it is shown that strains of *D. funebris* from different parts of the world are easily crossed with each other. The salivary chromosomes of the hybrids are investigated for inversions and other chromosomal rearrangements. Only one type of long inversion is found, namely in chromosome 2 within some of the strains (PERJE, 1954). DUBININ and TINIAKOV (1945 and 1946) have made extensive studies on ecological races of *D. funebris* which differ in the relative frequencies of inversions in some of their chromosomes, showing that inversion heterozygotes are common in nature. In spite of the frequency of inversions in those populations the same basic gene arrangement is found in all the strains investigated by PERJE (1954), indicating that inversion homozygotes have not been able to stabilize in nature. As the cytological examination was not carried out immediately after capture of the flies, the lack of long inversion heterozygotes (except in chromosome 2) in these strains may depend on inversions being selected against in the small laboratory cultures.

Differences in the gene pool of the strains are demonstrated for modifiers

to the *scute* mutation (PERJE, 1955 a) and for lethals affecting the sex ratio (PERJE, 1955 b).

The present investigation was begun with the object of determining whether variations in the pairing affinity of salivary chromosomes in hybrids occurred and whether the extent of synapsis could be regarded as an indicator of the relationships between the strains. First, synapsis of the chromosomes within the strains and in the crosses between the strains was analyzed. Secondly, as a consequence of the first investigation a more detailed examination of synapsis in the heterochromatic part of the X was carried out. Thirdly, some small inversions within the strains were analyzed.

II. MATERIAL AND METHODS

The cytological preparations of the salivary gland chromosomes were made in the same way and were analyzed in a phase microscope with the same equipment as described by PERJE (1954). As E. GOLDSCHMIDT (1952) has pointed out that synapsis of the salivary chromosomes is influenced by temperature and by the age of larvae, the precaution was taken to breed the larvae in a constant temperature room at 25°C and to dissect them some hours before the pupal stage. The results within the strains can serve partly as controls.

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Within each experiment 50 chromosomes of each kind were analyzed and their asynaptic regions noted. Only female larvae were used and the 50 chromosomes were taken from 3 different preparations in order partly to avoid individual differences being classified as strain differences. The synapsis of chromosomes 2 was examined in preparations without the inversion earlier described.

In the experiments on synapsis in the heterochromatic regions of the X-chromosome, as many heterochromatic sections as possible were investigated in three preparations of each strain and each type of hybrid.

The lengths of the asynaptic regions were determined by measuring the length of the corresponding segment in an arbitrary unit system on the standard chromosome map (PERJE, 1954).

III. RESULTS

A. ANALYSIS OF ASYNAPSIS IN STRAINS AND CROSSES

From each strain and from each cross with the L strain 50 chromosomes were checked for asynaptic regions. The results are given in table I. All the chromosomes are mainly paired and only a few exceptions are found. Most of the unpaired regions are localized in the X-chromosomes. Chromosomes 4 and 5 are the most stable. As seen from the totals there are more asynaptic

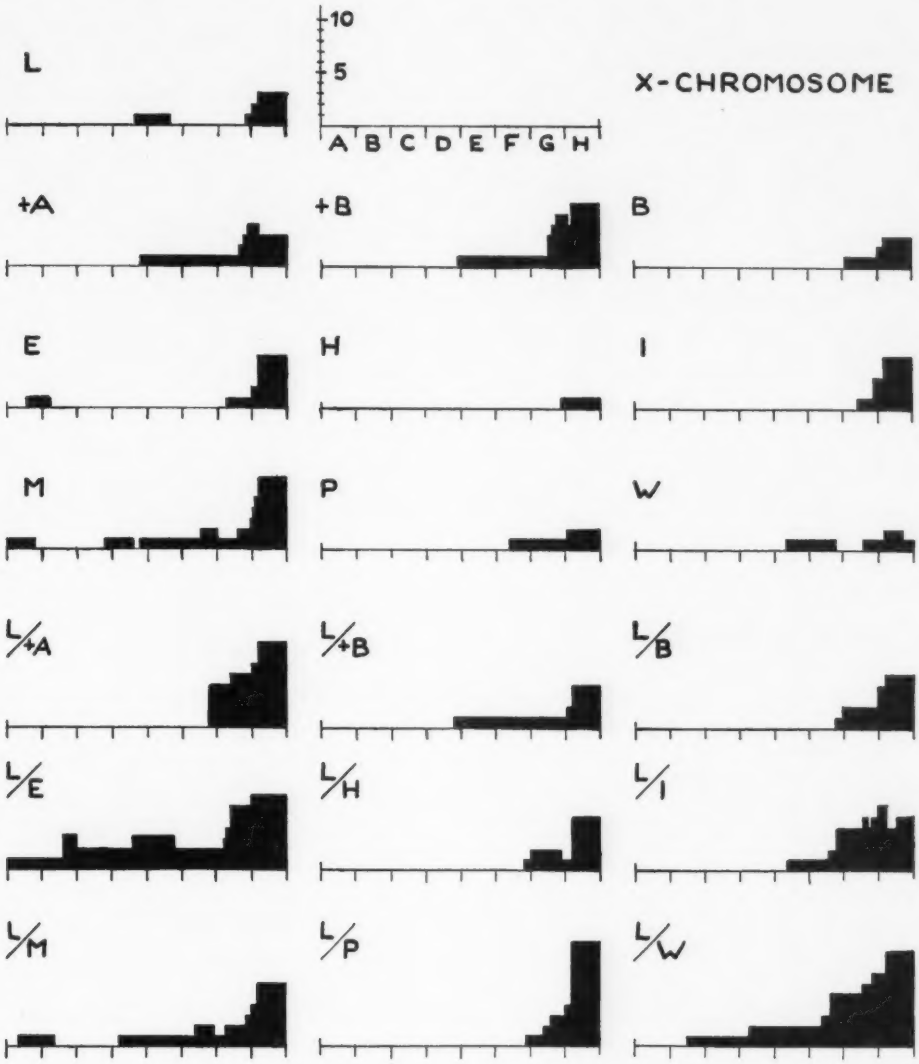

Fig. 1. Localization of asynaptic regions in the X-chromosome of strains and of crosses with the L strain in *D. funebris*. The black regions in the X-chromosomes represent the number of chromosomes showing asynapsis out of 50 chromosomes investigated.

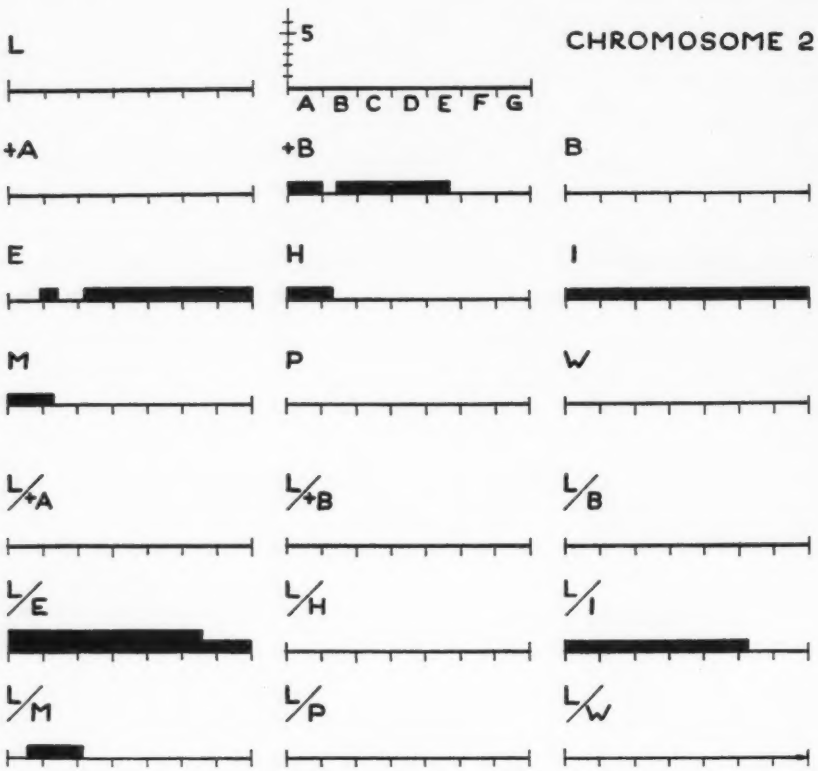

Fig. 2. Asynaptic regions in chromosome 2.

TABLE I. Asynapsis in different strains and in crosses with the L strain in *D. funebris*.

Strains	Percentages of asynaptic regions in chromosomes									
	X		2		3		4		5	
	Pure strains	Crosses with L	Pure strains	Crosses with L	Pure strains	Crosses with L	Pure strains	Crosses with L	Pure strains	Crosses with L
L	1.11	—	0	—	1.86	—	0.04	—	0	—
+ A	1.93	3.63	0	0	0	0	0	0	0	0
+ B	2.80	1.90	1.26	0	0	2.00	0	0	0	0
B	1.07	2.02	0	0	0	0	0	0	0	0
E	1.80	6.01	1.60	3.56	2.72	1.33	0	1.21	0.19	0
H	0.33	1.81	0.34	0	0	1.19	0	0	0	1.39
I	1.91	3.20	2.00	1.24	0	2.83	0	0	0	0.19
M	3.41	3.27	0.33	0.43	2.00	2.84	0.43	0	0.30	0
P	0.74	3.23	0	0	2.00	0	0	0	0	0
W	0.88	6.24	0	0	0.49	0	0	0	0	0
Total	1.60	3.48	0.55	0.58	0.91	1.13	0.05	0.13	0.05	0.18

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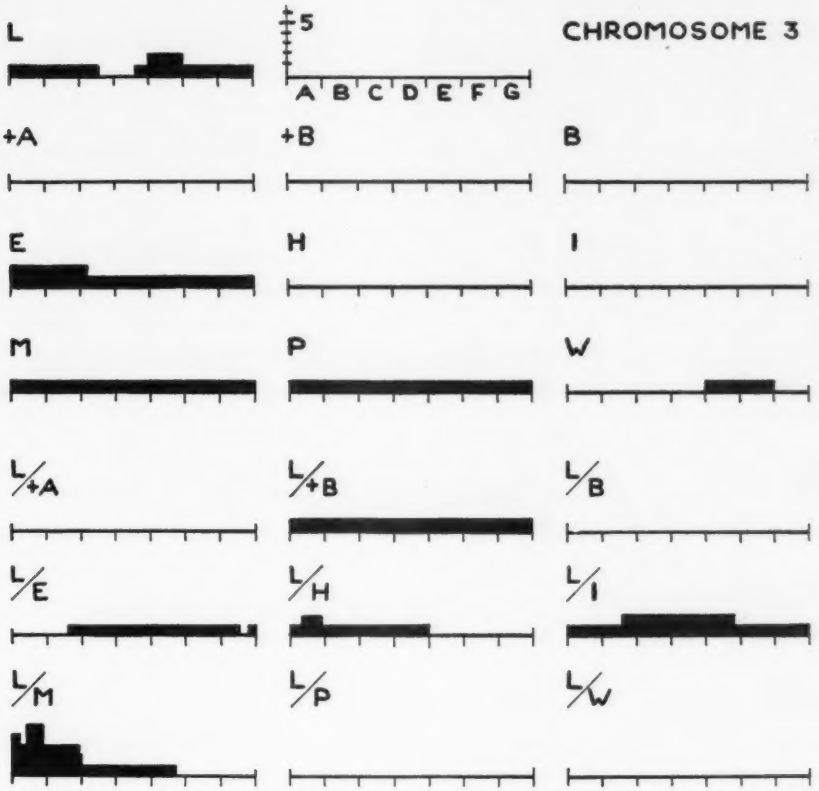

Fig. 3. Asynaptic regions in chromosome 3.

regions in the crosses than in the pure strains. In the autosomes the differences are too small to determine whether the disturbances in pairing between the partners should depend on genetical conditions or on mere chance. As for the X-chromosome, it is obvious that the pairing tendencies are decreased in the crosses except in the +B and the M strains, where the disturbances are rather high even in the pure strains. The arrangement of the asynaptic regions are shown in figs. 1—5, indicating that the distribution is random in the autosomes but mainly localized to the proximal heterochromatic region of the X-chromosome. Possibly these irregularities in pairing depend on disturbances caused by the formation of the nucleolus, as the nucleolus-organizer is situated to the right of H 1 in the chromosome map (PERJE, 1954). If this is the case the disturbances should be distributed at random, as no differences have been found in the size of the nucleolus in the strains and crosses. Another explanation would be that the heterochromatic parts are more easily separated from

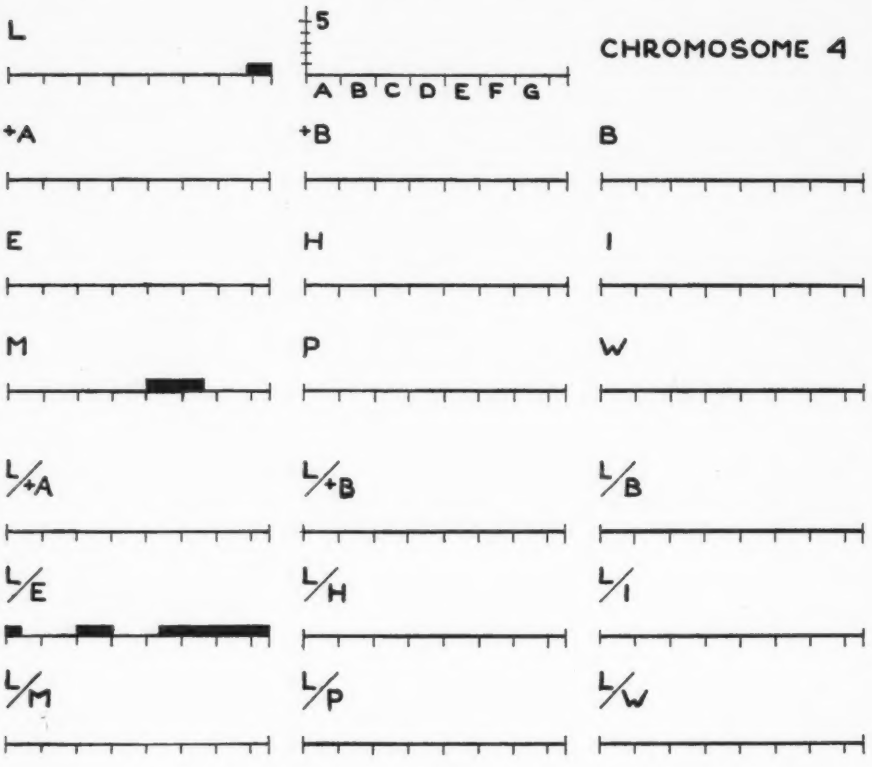

Fig. 4. Asynaptic regions in chromosome 4.

TABLE II. Analysis of asynapsis in the proximal heterochromatic region of the X-chromosome in different strains of *D. funebris*.

Strains	Number of X-chromosomes with the proximal heterochromatic region	
	Paired	Unpaired
L	296	9
+ A	187	12
+ B	202	18
B	211	11
E	185	9
H	57	1
I	270	10
M	132	9
P	99	4
W	123	3

χ^2 of heterogeneity 13.48
Degrees of freedom 9
P 0.10—0.20

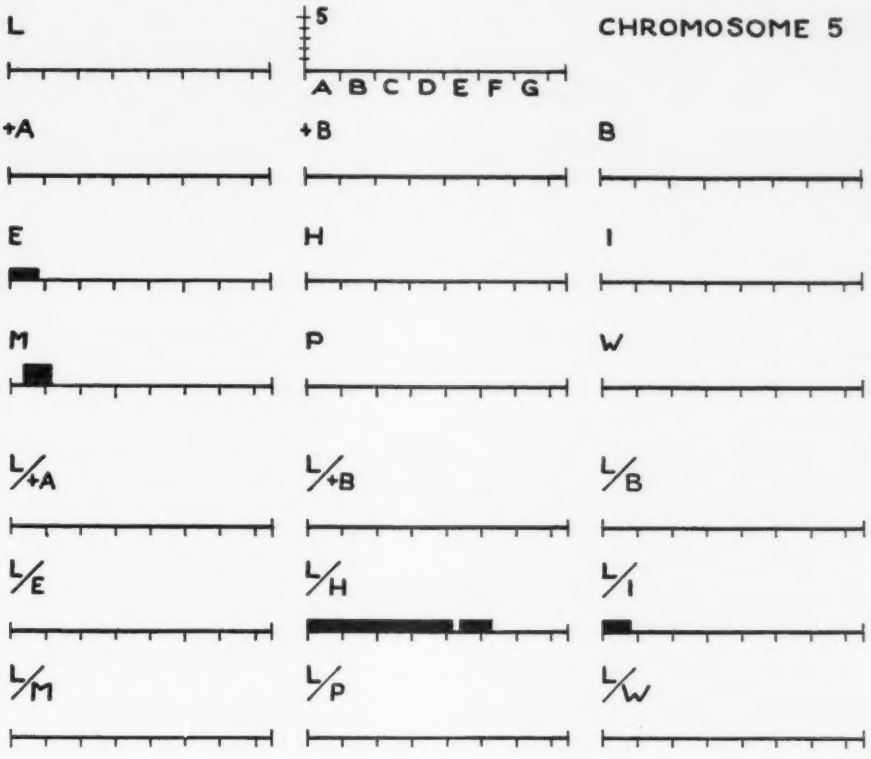

Fig. 5. Asynaptic regions in chromosome 5.

each other by mechanical forces in producing the cytological preparations. The chromosomes in the salivary glands are united in a chromocenter, which is much more easily destroyed than in *D. melanogaster*. Micromanipulation has shown that the chromosomes can easily be separated from each other at the chromocenter, but that it has been impossible to split up the proximal heterochromatic parts of the X-chromosome into its two homologues. This indicates that the irregularities in pairing are not due to the preparation methods.

To determine whether there are real differences between strains and hybrids in the pairing of the heterochromatic regions a more thorough investigation was carried out. In three cytological preparations from each strain and the reciprocal crosses to the L strain all cells that could be analyzed, were examined for paired (figs. 6 and 7) or unpaired (figs. 8 and 9) heterochromatin in the X-chromosome. In table II the results from the strains are given. A χ^2-test of heterogeneity gave $\chi^2 = 13.48$ for nine degrees of freedom, which corresponds to a P-value between 0.10 and 0.20. Thus no real differences between the strains are found and the asynapsis may depend on a split from

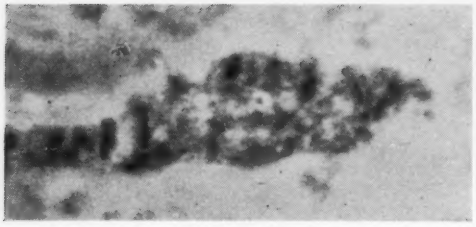

Fig. 6.

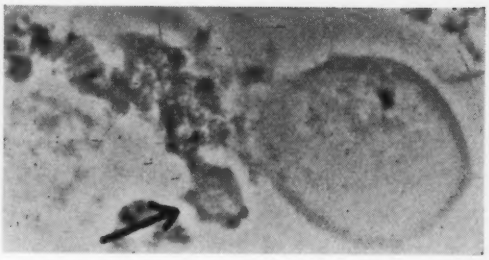

Fig. 7.

Fig. 6. Proximal heterochromatic part of the X-chromosome in the salivary glands of *D. funebris*. 960 ×. Phase microscope.
Fig. 7. Proximal heterochromatic part of the X-chromosome with the nucleolus. The arrow indicates chromosome 6. 720 ×. Phase microscope.

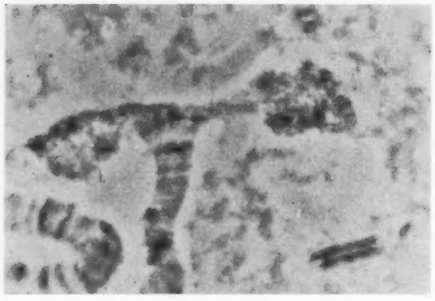

Fig. 8.

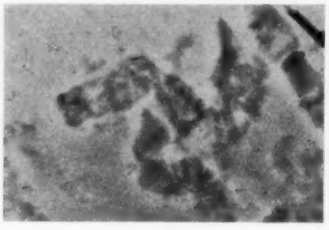

Fig. 9.

Figs. 8 and 9. The heterochromatic part of the X-chromosome in asynapsis. 720 ×. Phase microscope.

the nucleolus. In table III the conditions in the crosses are shown. A χ_c^2-test (χ^2 with Yates' correction for continuity) between the total for the pure strains and the crosses give $\chi_c^2 = 40.64$ with $P < 0.001$. Thus the asynapsis in the heterochromatin of the X-chromosome is significantly more frequent in the crosses than in the strains. χ_c^2-tests of the results from reciprocal crosses (table III) give good agreement in most crosses, the probability being less than 0.001 only in the crosses with the W and the P strains. In another χ_c^2-analysis the crosses are tested against pooled values of corresponding parental strains (table III, the last two columns). Twelve of the eighteen P-values have $P < 0.05$ which indicates real differences between crosses and their parental strains.

B. SHORT INVERSIONS WITHIN THE STRAINS

Berrie and Sansome (1948) have described inversion heterozygotes for chromosome 5 in a natural population of *D. funebris* from Manchester. Of

TABLE III. Analysis of asynapsis in the proximal heterochromatic region of the X-chromosome in crosses with the L strain in *D. funebris*. χ_c^2-test of homogeneity is made for reciprocal crosses. Another χ_c^2-test is carried out between crosses and the pooled values of parental strains.

Crosses	Number of X-chromosomes with the proximal heterochromatic region		χ_c^2 of homogeneity between reciprocal crosses	P	χ_c^2 between crosses and the pooled values of parental strains	P
	Paired	Unpaired				
L/+A	289	18	0.00	> 0.95	0.86	0.30—0.50
+A/L	207	13			0.69	0.30—0.50
L/+B	163	26	3.53	0.05—0.10	13.78	< 0.001
+B/L	236	20			1.72	0.10—0.20
L/B	114	31	3.48	0.05—0.10	47.66	< 0.001
B/L	132	19			15.14	< 0.001
L/E	291	37	1.53	0.20—0.30	17.55	< 0.001
E/L	68	4			0.23	0.50—0.70
L/H	127	5	0.74	0.30—0.50	0.09	0.70—0.80
H/L	325	22			4.82	0.02—0.05
L/I	317	23	0.57	0.30—0.50	5.35	0.02—0.05
I/L	331	31			11.59	< 0.001
L/M	204	22	0.03	0.80—0.90	7.71	0.001—0.01
M/L	87	10			5.19	0.02—0.05
L/P	386	19	12.69	< 0.001	0.85	0.30—0.50
P/L	238	34			20.58	< 0.001
L/W	280	72	19.78	< 0.001	61.34	< 0.001
W/L	205	14			4.03	0.02—0.05

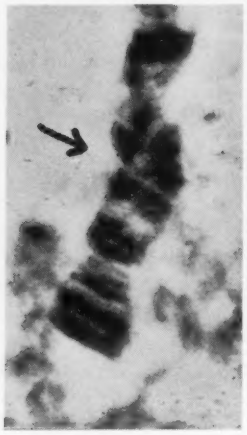

Fig. 10.

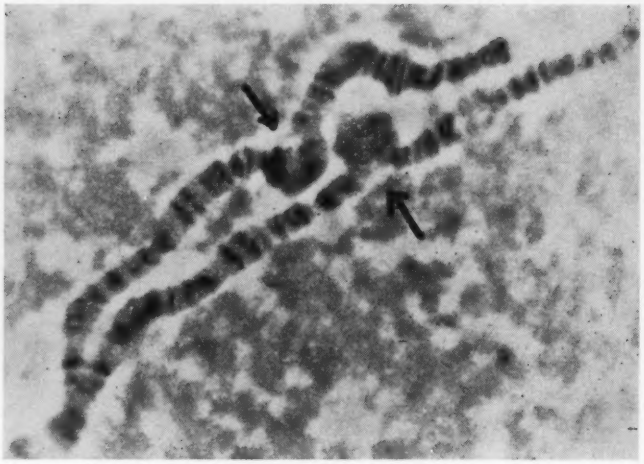

Fig. 11.

Fig. 10. A small inversion in A5 in chromosome 5. 1600 ×. Phase microscope.
Fig. 11. The unpaired distal end of chromosome 4 showing pairing of duplicate segments in B3—5. 720 ×. Phase microscope.

Fig. 12.

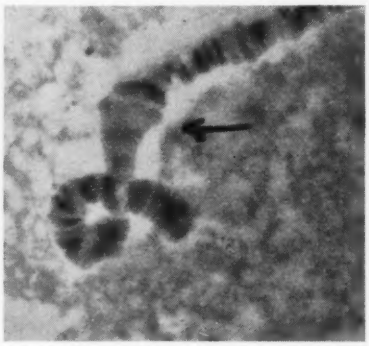

Fig. 13.

Fig. 12. Duplication 4B3—5 as an inversion loop. 720 ×. Phase microscope.
Fig. 13. Chromosome 4 with normal pairing in the segment 4B3—5. 720 ×. Phase microscope.

40 flies investigated all were heterozygous for the inversions. Cytological investigations of the strains used in these experiments showed that the inversion in 5A5 occurred in some percentages of the cells of all larvae investigated in both strains and crosses (fig. 10). Other cells within the same glands did not show the inversion loop, even if the preparation was very distinct for the bands

in question. A possible explanation of this phenomenon is that a duplication of the combination *abba* has arisen. In such a case the pairing can either be normal or form an inversion loop. Another explanation, suggested by BERRIE and SANSOME (1948) is that the species has built up a balanced system to hold the inversion in heterozygous condition. The latter hypothesis cannot give an explanation of the normal pairing in some of the cells. Another inversion occurring in most of the cells is the 4B3—5 duplication, mentioned by PERJE (1954). In this case it is obvious that pairing takes place even if the homologues are not paired (fig. 11). Sometimes there is an obvious inversion loop, in other cases the chromosome is only folded by the pairing of segments within the same chromosome (fig. 12). Often the upper part of the inversion loop or duplication puff is more or less heterochromatic without distinct bands. In about 12 % of the 950 cells investigated no inversion loop or puff is seen and the homologous chromosomes show normal pairing (fig. 13).

IV. DISCUSSION

Usually heterochromatic regions are considered to have non-homologous pairing. The proximal heterochromatic parts of the X-chromosomes of *D. funebris* have been shown to have different pairing tendencies in crosses between different strains. Such increased asynapsis in the hybrids indicates that the pairing is at least partly homologous, as related chromosomes pair more easily than chromosomes from populations that have developed in different directions for a long time. As this region of the X-chromosome is the Y-homologous section which has been shown to have a high frequency of lethals (PERJE, 1955 b), it is already determined genetically that these parts of the chromosomes have developed quite different balanced lethal systems, probably also directed by the autosomes or some other part of the X-chromosome.

The small inversion loops found in chromosome 4 and 5 in all the larvae investigated indicate either a highly balanced system for inversion heterozygotes, or duplications. Both these systems further heterozygous conditions; the latter by the possibility of building up balanced lethal systems and modifiers within the duplication (PERJE, 1955 b).

As one of the most prominent features of *D. funebris* is the large pool of variability concealed in the strains and shown by the variable penetrance and low expressivity of many known mutants, is is obvious that some balanced genic mechanism must function to retain this gene pool in the populations.

V. SUMMARY

1. Asynapsis of the salivary chromosomes of *D. funebris* is analyzed in 50 chromosomes of each kind in 10 strains and in 9 crosses with the L strain. The percentage of asynapsis is calculated by measuring the distances in the standard map of the salivary chromosomes (PERJE, 1954). Diagrams of the asynaptic regions are given. In the X-chromosome asynapsis is greater in the crosses than in the pure strains. It is mainly localized in the proximal heterochromatic region. The autosomes show very intimate pairing in both the strains and crosses.

2. A more thorough investigation of the pairing tendencies in the proximal heterochromatic section of the X-chromosome shows that the partners are more often unpaired in the crosses than in the pure strains. This indicates homologous pairing of the heterochromatic regions.

3. Short inversions, probably depending on duplications of the type *abba* are described for chromosomes 4 and 5.

4. These duplications or inversion heterozygotes are assumed to aid the species to retain a great variability in the gene pool, a condition which is typical for the species.

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ON THE STRUCTURE AND FUNCTION OF THE ACCESSORY OLFACTORY ORGAN IN LAMPREYS

BY

LARS-OLOF HAGELIN

and

ALF G. JOHNELS

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1. INTRODUCTION

The accessory olfactory organ (DE BEER 1925, LEACH 1951, "Folliculäre Anhänge" MATTHES 1934) in adult lampreys consists of a large number of small epithelial vesicles, aggregations of which are distributed behind the caudal parts of the folds of olfactory epithelium bordering the nasal cavity (cf. fig. 1 a). These vesicles lie in an extremely vascular connective tissue within the caudal space of the cartilaginous olfactory capsule. The aggregations of vesicles are particularly voluminous in the ventro-caudal part of the olfactory capsule. In this place a few vesicles are formed as diverticulae from the olfactory epithelium already in young larvae (SCOTT 1887, cf. also DE BEER 1923, 1925). The organ develops only slightly during larval life. During metamorphosis the two simple sacs of olfactory epithelium, forming the olfactory organ of the larva, are changed into a complicated structure with many folds in each sac. The perfection of the accessory organ takes place during fairly late metamorphic stages by the development of an aggregation of vesicles behind each of these folds (cf. e.g. LUBOSCH 1905, DE BEER 1925, IMAMURA 1928).

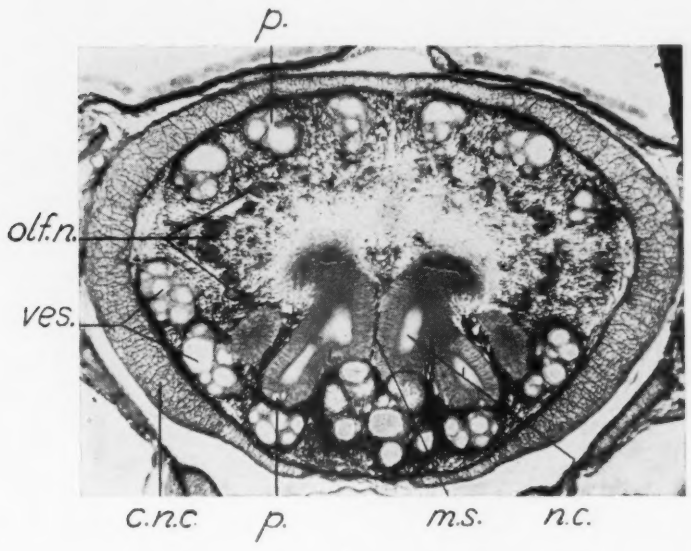
In the literature there are many conflicting opinions regarding the function of this accessory organ, but the various authors may be put into either of two main groups. The first consists of those who believe the function to be sensory and the second of those who consider it to be glandular.

The first group seems to include only two authors. SCOTT (1887) — the first to observe the existence of the accessory olfactory organ — suggested that it could be homologous with Jacobson's organ of higher vertebrates. LUBOSCH (1905) regarded the organ as degenerated caudal parts of olfactory sacs which had been more extensively developed in earlier stages of evolution.

A larger number of authors consider the organ to be glandular but the ideas regarding the nature of the secretion vary considerably. Many believe it to be exocrine, the secretion entering the cavity of the olfactory organ through a number of minute excretory ducts (e.g. POGOJEFF 1888, KAENSCHE 1890, BALLOWITZ 1904 a, BÜTSCHLI 1921, KRAUSE 1923, LEACH 1951). WOERDEMAN (1915) suggested that the accessory organ in lampreys might be homologous with the lobuli laterales of the hypophysis in higher vertebrates. Other homologues proposed are the lateral nasal glands of *Amphiuma* and Bowman's glands in mammals and man (cf. DE BEER 1925, LEACH 1951). DE BEER considered the organ to be of an endocrine nature (cf. also TRETJAKOFF 1927) partly because of the intimate relationship to the surrounding blood vessels. Another reason for this idea was, however, that in his opinion there were no communications in the adult between the vesicles and any other cavity. The same was stated also by SCOTT (1887), VOGT & YUNG (1889—94), LUBOSCH (1905), and IMAMURA (1928).

The histological structure of the wall of the accessory olfactory organ has been described by POGOJEFF (1888), BALLOWITZ (1904 b), LUBOSCH (1905), KRAUSE (1923) and DE BEER (1925). From the works of these authors it may be gathered that there are two kinds of cells in the vesicular walls: one a large type and the other a smaller consisting of interstitial cells which surround the former. DE BEER discussed in great detail the fact that the large type of cells could be regarded as glandular although these cells possess cilia, directed into the cavity of the vesicles. LUBOSCH (1905), DE BEER (1925), LEACH (1951) and others have observed that the aggregations of vesicles of the accessory organ are innervated by branches running along the olfactory nerve, believed to be motor by LEACH. From LUBOSCH's and DE BEER's descriptions it appears that the vesicles of the accessory organ develop by budding from the olfactory epithelium of the nasal sacs.

From what has been quoted above the following three questions may be said to arise: (1) Are there communications between the vesicles of the accessory olfactory organ and the nasal cavity, or not? (2) What kind of nerve fibres form the innervation of this accessory organ? (3) What is the histological structure of the walls of the vesicles? It seems that answers to these questions would form a basis for a solution of the problem of the function of the accessory olfactory organ in lampreys.



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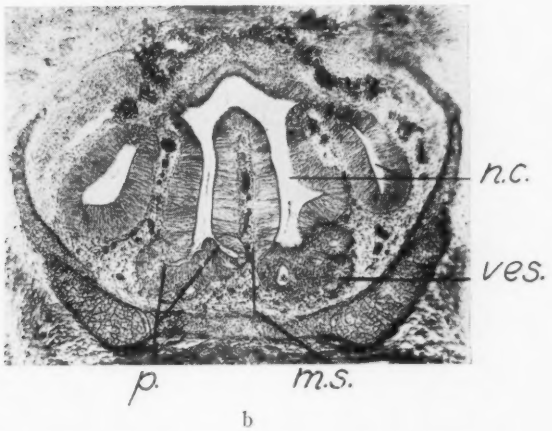

b

Fig. 1 a. Transverse section through the caudal portion of the nasal capsule of adult *Lampetra planeri*. Groups of vesicles of the accessory olfactory organ are seen inside the cartilaginous wall. 50 x. b. Horizontal section through ventral part of nasal capsule of *Lampetra planeri* in metamorphosis. Lateral folds are developing. On each side of the base of the median nasal septum a few vesicles of the accessory organ are seen which still in the main retain the larval organization. 55 x. Microphotographs. c.n.c. — cartilaginous nasal capsule. m.s. — median nasal septum. n.c. — nasal cavity. olf.n. — olfactory nerve. p. — pore. ves. — vesicles.

2. MATERIAL AND METHODS

The present studies were made on material of *Lampetra planeri* and *L. fluviatilis* which was fixed in Bouin's fluid (Romeis 1948), Bodian's fixative no. 2 (Bodian 1937) and according to Smith (1912). Particular attention was paid to the fixation because of the fact that the accessory olfactory organ has a very protected position and, in addition, is surrounded by dense accumulations of blood cells which aid very effectively in delaying the penetration of the fixatives. The material was embedded in paraffin and sectioned with a thickness of between 6 and 15 microns in the slides. The staining was Heidenhain's iron haematoxylin, Heidenhain's axan, and silver impregnations according to Bodian (1936) and Palmgren (1948). In addition the ontogenetic and metamorphic development of the organ was studied on a large number of preparations (cf. Johnels 1948).

3. THE COMMUNICATION BETWEEN THE ACCESSORY OLFACTORY ORGAN AND THE NASAL CAVITY

As mentioned in the introduction the olfactory epithelium forms two sacs in the larva, incompletely separated in the vertical midline by a medial nasal septum. The ventral end of the sacs is slightly prolonged caudally and in this place a bundle of small vesicles forms the accessory olfactory organ of the larva (cf. fig. 1 b). As demonstrated by Lubosch (1905) these vesicles maintain the communication with the cavity of the nasal sac. De Beer (1925) failed to observe these connecting pores although he worked on the same species as Lubosch (*L. fluviatilis*, *L. planeri*). Leach (1951) studied American lampreys. According to him the accessory olfactory organ is represented only by an anlage before the metamorphosis. It is pictured, however, in a photograph of a cross section of a 42 mm larva of *Ichthyomyzon fossor*.

During metamorphosis the lateral sacs of the larval olfactory organ are highly transformed. In each sac there develop 7—10 folds (fig. 1 a) and at the caudal border of each fold a bunch of vesicles develops from the olfactory epithelium (Kaensche 1890, Lubosch 1905, Imamura 1928, and others). At the same time the rate of propagation of vesicles in the larval accessory organ (fig. 1 b) increases considerably and as a result the greatest aggregation of vesicles is situated in this place also in the adult. De Beer was of the opinion that there was a difference in mode of formation between the accessory organ in the larva and the other aggregations, formed during metamorphosis. This opinion was probably due to lack of relevant metamorphic stages. The process of formation is the same during metamorphosis as before: vesicles are formed

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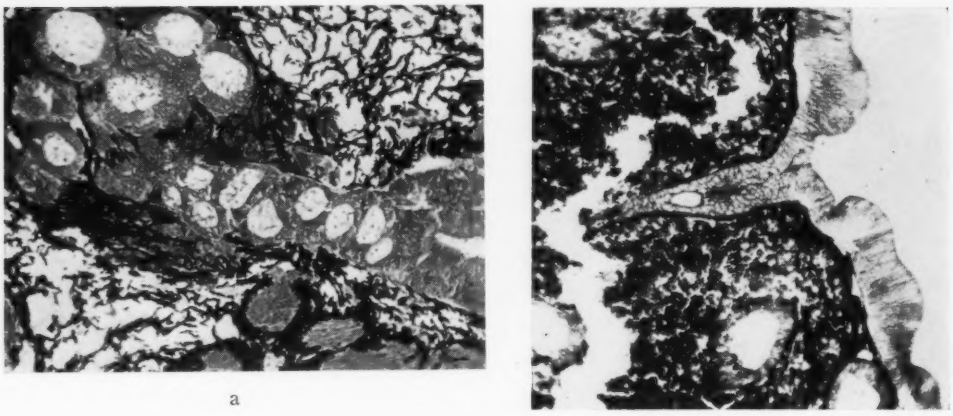
by proliferation from the olfactory epithelium, sink below the typical olfactory epithelium into the connective tissue and proceed to propagate into more vesicles. A few vesicles persist in close connection with the olfactory epithelium.

As a result of this process of formation there are, in the large *L. fluviatilis*, two very conspicuous ducts which open on each side on the ventral part of the median septum, forming the communication between the two large ventromedian aggregations of vesicles and the nasal cavity. The exterior part of these ducts consists of a columnar epithelium of on an average the same height as the indifferent epithelium covering the non-sensory parts of the nasal cavity. At some distance from the exterior opening the epithelium abruptly changes to a roughly cuboidal epithelium, which forms short ducts between the large duct and a number of vesicles (fig. 2 b). It seems clear, however, that by no means all vesicles have a direct connection with such ducts. Instead the vesicles have immediate communications with each other by tiny pores (noticed by DE BEER and others) and in this way form a system which is connected with the ducts.

In the somewhat smaller groups of vesicles at the caudal edge of the folds of olfactory epithelium which are formed — the folds as well as the vesicles — during metamorphosis the situation is slightly different. Usually there are very short ducts or hardly any distinct ducts at all formed in these places. The communication with the exterior may be formed by a row of vesicles (fig. 2 a), the most superficial of which opens on the surface of the olfactory epithelium. Often this communication has the character of a very tiny pore between the columnar cells of the olfactory epithelium. A group of vesicles may have more than one communication with the nasal cavity.

In a few cases it has been impossible to locate the connection with the exterior of a group of vesicles. In addition it has not been possible to establish that all vesicles in an aggregation really have a communication with a system which leads to the exterior. This is at least partly explained by the complexity of the organ, consisting of large numbers of vesicles in *L. fluviatilis*. Further, there is no peculiar differentiation of the cells in the places where the tiny pores penetrate the walls of adjacent vesicles. Therefore the existence of these pores may easily be overlooked if they are not cut at a favourable angle (longitudinally, or almost so) and particularly in too thick sections (10 microns or more). There are also other circumstances to be taken into consideration in this connection (cf. p. 122). Often the vesicles are not rounded or ovoid structures as most authors say but are tubular in shape as noticed by LEACH (1951).

LEACH (1951) considers that the accessory olfactory organ exhibits a relatively greater development in brook lampreys than in predatory ones. The contrary is true in the present material. The number of vesicles is much larger in the predatory *L. fluviatilis* than in the non-predatory *L. planeri*. Although



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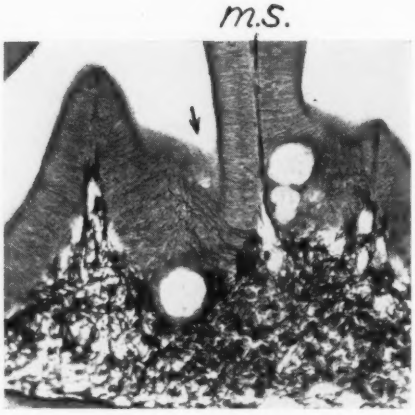

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Fig. 2 a and b. *Lampetra fluviatilis*, sagittal sections of nasal capsule. a. Row of vesicles forming connection with nasal cavity, situated to the right in the figure. Note moderate number of blood cells close to the vesicular walls among the scattered pigment cells and cut branches of olfactory nerve in lower part of the picture. b. Connecting duct between vesicles and nasal cavity. Blood cells in large numbers completely surround duct and vesicles. Rupture in the blood cell mass is an artefact. c. Transverse section through the connecting structures between the nasal cavity and the ventro-median aggregation of vesicles of adult *Lampetra planeri*. The arrow points to pore opening on the surface of the olfactory epithelium. Large pores within olfactory epithelium are seen to the right on the median septum (m.s.). A moderate number of blood cells are seen among the pigment cells. — Microphotographs, 120 x.

the same structures have been noticed in *L. planeri* as in *L. fluviatilis*, observations are rendered more difficult in the former species because of the smaller size of the details. The very obvious openings of the large ventral ducts in *L. fluviatilis* are often visible only as small pores in the olfactory epithelium of *L. planeri* (fig. 2 c).

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There are, thus, distinct structures which connect the interior of certain vesicles of the accessory olfactory organ with the nasal cavity. In addition it has been verified that direct communications exist between vesicles of the organ. It seems justified to suppose that all vesicles communicate directly or indirectly with the nasal cavities.

4. THE INNERVATION OF THE ACCESSORY OLFACTORY ORGAN

In all vertebrates the olfactory nerve is formed by cell processes belonging to primary sense cells, the olfactory cells, which take part in the formation of the olfactory epithelium. Consequently, this nerve has a very typical structure which is different from that of other peripheral cranial nerves. A distinction between true olfactory fibres and fibres of some other nature therefore seems to be easily discernible.

In preparations which have been impregnated with silver according to BODIAN (1936, 1937) or PALMGREN (1948) the staining showed no difference whatsoever between the fibres which approach the accessory organ and those coming from the olfactory epithelium. In no case was the impregnation of the olfactory fibres really selective but it was tolerably distinct. TRETJAKOFF (1927) mentions that the selective staining of nervous elements in the vicinity of the accessory olfactory organ was obstructed by the presence of large amounts of blood (cf. below). However, other peripheral nerve fibres as well as fibres in the brain were selectively stained in the same slides. In addition it was ascertained by tests that the applied methods selectively stained autonomous fibres in other regions of the body. In the preparations all fibres in the olfactory nerve are histologically identical. It seems reasonable to conclude that, if the fibres of the accessory olfactory organ had been of a principally different nature from the rest of the olfactory fibres, this would have been demonstrated in the preparations. In a few cases fibres from the accessory olfactory organ branch have been followed up to their origination in cells within the wall of a vesicle.

5. THE HISTOLOGY OF THE VESICULAR WALLS

The studies by LUBOSCH (1905) and DE BEER (1925) agree as regards the fact that there are two different kinds of cells in the epithelium wall of the vesicles. The large type is described by LUBOSCH (1905 p. 122) as follows: "sie hatte die Form einer dickbäuchigen, kurzhalsigen Flasche und zeignete sich durch etwas intensivere Färbung aus." He failed, however, to find these

cells in all vesicles and the same was the case with the cilia. De Beer noticed the general presence of cilia on this large type of cell and the distribution of this kind of cell in the walls of the vesicles.

In the introduction the difficulties of proper fixation of these structures were pointed out. It seems that the somewhat fragmentary and conflicting observations of earlier authors (cf. also Pogojeff 1888, Krause 1923, Leach 1951) may be explained by that fact. The shape of the cilia and the distal part of the large cells carrying them seem to be particularly influenced by improper fixation (cf. also p. 122). De Beer (1925) noticed a coagulated fluid in the vesicles which he believed to penetrate between the cells to the exterior blood vessels. It seems that this fluid is a product of the fixing process. The same has been observed in the nasal cavity, in the naso-pharyngeal pouch, and often also in the buccal cavity.

In slides impregnated according to Palmgren (1948; the result is somewhat capricious, which may be regarded as a normal feature of silver impregnations) the cilia appear distinctly as tufts (fig. 3 a—d) placed on the top of eminences formed by the distal parts of large cells which protrude slightly above the surface of the epithelium. The rest of the cell body of the large cells is embedded in the epithelium. The shape of these cells is rounded or ovoid depending on the thickness of the epithelium, which varies considerably. The nucleus usually has a basal position, remote from the surface. In preparations stained with iron haematoxylin the same structures are visible; the cell bodies become densely stained as observed by Lubosch (cf. quotation above). In a few cases the basal part of the large cells has been observed to proceed in a thin process which joins a bundle of similar fibres. These fibres run towards the olfactory nerve. The large cells exhibit striking similarities to the sense cells of the olfactory epithelium which have been described by Retzius (1880) and Ballowitz (1904 b, cf. fig. 3 e), and the present authors consequently conclude that the large cells are olfactory sense cells.

The supporting cells form an interstitial system which separates the large cells from each other. The plasm of the supporting cells appears to be very dense as noticed by De Beer. Particularly at the surface a cover is formed in which small areas are left free for the distal, ciliated eminences from the large cells. There are no cilia on the supporting cells in the accessory organ. Apart from this the supporting cells and surface differentiations are of the same type as in the olfactory epithelium.

There are important similarities between the structure of the walls of the vesicles and the olfactory epithelium from which the former structures originated. It seems that the difference in the shape of the cellular elements is caused by the different depth of the two kinds of epithelium. However, the difference in shape is small as regards the sense cells because these cells in

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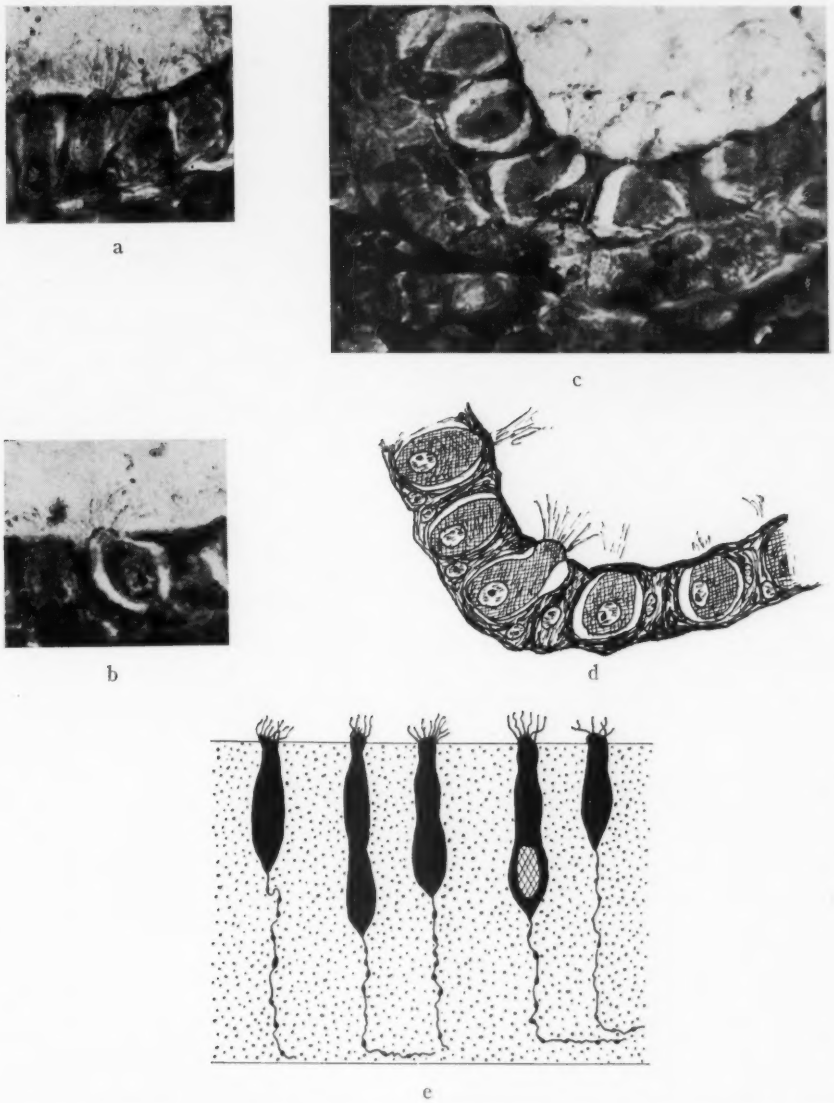

Fig. 3. *Lampetra fluviatilis*. a and b. Sense cells in the wall of a vesicle. In a. cilia of sense cells and surface cover formed by supporting cells are particularly obvious, in b. the basal granules of the cilia are seen as black dots forming a small curve along the protruding eminence of the sense cell. c and d. The composition of the wall of the vesicles. Note in c. the blood cells closely packed together outside the vesicular wall between branched pigment cells. The drawing d is constructed from two consecutive sections one of which is pictured in c. Sense cells cross-hatched in the drawing between non-ciliated supporting cells. e. Sense cells in olfactory epithelium (after BALLOWITZ 1904 b). a—c microphotographs. d and e drawings. a—e 750 x.

the olfactory epithelium of the nasal cavity occupy only a part of the total epithelial depth, if the cellular process is not taken into consideration (cf. fig. 3 e).

6. DISCUSSION

It seems clear that to an organ which has typical olfactory cells in connection with olfactory nerve fibres must be ascribed an olfactory function. It is equally evident that an olfactory organ must have a communication with the exterior for a normal function. This contact may seem to be very limited and inefficient in the case of the accessory olfactory organ.

In the present material the vesicles mostly appear to be rather constricted in the preparations. Often there is a considerable space between the wall of the vesicles and the surrounding connective tissue, which is densely filled up by blood cells (cf. fig. 1 a, 2 b and 3 c). It is impossible to observe the walls of the blood sinuses directly because of the enormous accumulation of blood cells, but their approximate distribution may be tolerably identified because of the pigment cells which are extremely common in the tissues in question (cf. e.g. KRAUSE 1923).

DE BEER (1925) attached great importance to the vascularity of the accessory olfactory organ. Its strong development was considered to be sufficiently explained only in functioning as a receiving system of the supposed endocrine products of the organ. Apart from the fact that also exocrine glands may require a strong supply of blood vessels, there seems to be another possibility of explaining the presence of the blood sinuses, and particularly their strong development.

The tissue in the vicinity of the vesicles has the character of a cavernous tissue serving as a mechanism for changing the volume of the vesicles. When the blood accumulates in the surrounding sinuses the vesicles become constricted and partly emptied of their contents. If, on the other hand, the blood in the sinuses is drained off, the vesicles will expand and consequently become refilled with contents from the nasal cavity. It is possible that the tension of the olfactory epithelium of the nasal sacs also is influenced by this process.

This change in the volume of the vesicles affects the shape of the cells of the walls. Very probably also the size of the pores between the vesicles as well as of the cavity of the connecting ducts (cf. fig. 2 b and p. 117) is influenced by this pumping mechanism. The inconsiderable size of the pores in the preparations is most probably due to the fact that the animals, when submerged in the fixative, try to empty the nasal cavities as far as possible. It seems, in addition, that this contracted state of the accessory organ is already caused when the animals are kept in tap water which has been chlorinated.

The presence of large blood sinuses is a normal feature in the head of

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lampreys (TRETJAKOFF 1926). It has not been possible to demonstrate in the preparations any kind of valve mechanism in the sinuses of the nasal capsule because of the large amount of blood cells. However, this accumulated blood mass seems to be as large in specimens which were decapitated before being fixed as in fixed intact specimens. This indicates the presence of valves which prevent the blood from being drained off. Besides, valves have been observed in other sinuses of the head by the present authors.

The homology between the accessory olfactory organ in lampreys and Jacobson's organ in higher vertebrates suggested by SCOTT (1887) has been rejected repeatedly (VOGT & YUNG 1889—94, LUBOSCH 1905, DE BEER 1925, LEACH 1951). It appears that this attempted homology throws a bridge across too large a distance without a sufficient number of interjacent piers. However, it may be interesting to note that (1) in both cases it is a question of a detached and specialized part of the olfactory organ. (2) In both cases this part is innervated by branches from the olfactory nerve and is (3) surrounded by cavernous supporting tissue. (4) The olfactory function of Jacobson's organ is of a special kind and different from the rest of the olfactory organ. Most probably the same is true of the accessory olfactory organ in lampreys.

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7. SUMMARY

The following observations on the structure of the accessory olfactory organ have been made:

1. There are distinct structures which connect the interior of vesicles of the accessory olfactory organ with the nasal cavity.
2. The nerve fibres of the olfactory nerve which supply the accessory olfactory organ and the rest of the olfactory nerve appear to be histologically identical.
3. The wall of the vesicles of the accessory olfactory organ consists of two kinds of cells. One type appears to be identical with the sense cells of the olfactory epithelium. The other type consists of supporting cells which are similar to the supporting cells of the olfactory epithelium. However, they lack cilia on the surface.
4. The connective tissue surrounding the vesicles of the accessory organ is extremely vascular and is regarded as a cavernous tissue, forming a pump mechanism which empties and fills the vesicles with fluid from the olfactory cavity.

It is concluded that the function of the accessory olfactory organ is related to the normal olfactory function but may be of a special kind.

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SKELETON OF CYPRINOID FISHES IN RELATION TO PHYLOGENETIC STUDIES 6. THE SKULL AND WEBERIAN APPARATUS IN THE SUBFAMILY GOBIONINAE (*CYPRINIDAE*)

BY

L. S. RAMASWAMI

(Department of Zoology, Univ. of Mysore, Central College, Bangalore, India.)

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INTRODUCTION

GÜNTHER (1868) was the first to visualise that the family Cyprinidae could be divided into groups or tribes. He accordingly split it into eleven groups of which Cyprinina comprised the largest number of genera.

REGAN (1911) while pointing out that the greatest number of cyprinid genera occurred in Asia, concluded that the characters exhibited by them were not sufficiently diagnostic to split them into subfamilies. He described the osteological characters of the family as follows:

"Premaxillaries excluding maxillaries from gape; Pharyngeal teeth, when present, in one, two, or three series, not more than seven in one series; pharyngeal processes of basioccipital typically united below the aorta to form a horizontal or oblique plate, flattish or concave below, supporting a horny pad, and produced backwards into a strong process for the attachment of the *retractor* muscles of the lower pharyngeals. Mesethmoid broad, firmly united with the frontals; subtemporal fossae deep; no temporal depressions, but subtemporal fossae more or less distinct, open behind, roofed by the post-temporal and sometimes by the pterotic and parietal. Cleithra normally suspended from supracleithra. Air-bladder divided into two parts by a constriction, typi-

cally large and free but sometimes reduced (*Discognathus* and *Gyrinochilus*), or the anterior part sometimes enclosed in a bony capsule formed by the *ossa suspensoria* (*Rhinogobio, Saurogobio*). Outer ramus of *os suspensorium* not connected with its fellow nor with the transverse process of second vertebra."

In describing the interrelationships of the genera, REGAN (1911) noted that "Günther's Cyprinina seems to be a natural group after excluding the North American genera, but to it should be added *Rhoteichthys* and *Osteobrama*, with the osteological characters of *Barbus*, and doubtless *Leptobarbus* and *Mystacoleucus* also; *Tinca* seems to be nearer to *Barbus* than to *Leuciscus*. The Rasborina and Danionina should be united and some of the Abramadina should be added to this group, some to the Leuciscins. *Xenocypris* is a Leuciscine, and the aberrant *Semiplotus* is, perhaps, nearest to it. *Hypophthalmichthys* is nearly related to the *Barilius* group".

WEBER and de BEAUFORT (1916) recognised three subfamilies in the cyprinid fishes of the Indo-Australian region: Abramadinae, Rasborinae and Cyprininae.

NICHOLS (1925) noted that the Yangtse valley was the center of distribution of the carp family and its members reached America through the Behring sea land-bridge. He (1936) divided the family into eight subfamilies, viz., Leuciscinae, Rasborinae, Abramadinae, Schizothoracinae, Rhodeinae, Cyprininae, Chondrostomatinae and Gobioninae based on purely external characters. He also reallocated several genera under different subfamilies, e.g., *Barilius* and *Danio* were taken away from Danionina and included under Rasborina. After making a comprehensive study of the fresh-water fishes of China, NICHOLS (1943) noted that it would not be wrong to suppose that the suckers were more primitive than carps and that the former have left only one survivor in China, viz., *Myxocyprinus* and have been superseded by the present day gudgeons, the near relatives of carps. The loaches which are not represented in America, are probably a secondary offshoot of the true carps. He cited *Gobiobotia* as a tangible evidence of the origin of loaches, which combines characters both of a loach and a gudgeon and included it under the Cobitidae.

MORI (1934) in describing the fish fauna of Jehol recorded *Gobiobotia*, *Saurogobio* (*dabrayi*) and *Microphysogobio* as Gobiobotinae while *Gobio*, *Hemibarbus, Gnathopogon, Pseudogobio, Abbottina* and *Pseudorasbora* are included under Cyprininae, the latter having groups like Barbini and Gobioninae.

In utilising the structure of the inferior pharyngeal bone and its dentition for the classification of the Cyprinidae, CHU (1935) remarked with regard to the subfamily Gobioninae, "Since the generic differentiations within this subfamily profusely involve other structures, the characters provided by the pharyngeals and teeth must supplement or be supplemented by those of other characters, such as body form, position of vent, the arrangement of the sense organs of the head, the mouth structures, the scale structure etc." With regard to the teeth, he remarked (p. 185), "The primitive type of teeth is believed

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to be one which is moderately compressed, in three rows (the main row of five teeth; second row of 4 teeth; third row of 2 teeth), with the tips more or less recurved and with the grinding surfaces narrow and rudimentary".

TARANETZ (1938) described that the gudgeons comprise of individuals with terminal and inferior mouths; the latter condition was developed in them by two different methods:

1) "Shifting of the mouth to a lower position and straightening out of the lower profile of the body" (*Armatogobio, Rostrogobio, Gobio*),

2) "Shortening of the lower jaw without any marked displacement of the mouth, and without straightening out of the lower profile of the body" (*Chilogobio, Sarcocheilichthys, Ladislavia*).

The evolution of the generalised forms like *Gnathopogon* and *Paraleucogobio* proceeded in two ways: *Chilogobio, Sarcocheilichthys* and *Ladislavia* developed the second type of mouth, sharpened the lower jaw, developed the ovipositor and reduced the barbels. Others developed the mouth of the first type, reduced their air-bladder and developed a capsule. Changes have taken place in the latter group: in one set, one row of pharyngeal teeth and an occipital foramen are noticed (*Saurogobio, Abbottina, Pseudogobio*) and in the other set, there is no occipital opening and the pharyngeal teeth are in two rows (*Gobio* etc.).

VASNECOV (1939) studied the development of the pharyngeal teeth in the Cyprinidae and traced the evolution of the various types from that in a primary form like *Leuciscus*. A figure is drawn to show the origin and evolution of teeth found in *Gobio, Cyprinus, Barbus, Carassius, Varicorhinus, Abramus* and *Chondrostoma* starting from the primary form. He also stated that "The manyrow teeth are primary for Eventognathi" thereby supporting REGAN (1908) and CHU (1935) but differing from BERG (1912).

BERG (1940) in describing the family Cyprinidae noted that it could be broadly divided into two groups: in the first the gill membrane fused with the isthmus, the suprabranchial organs were absent and the pharyngeal bones were *not* perforated. Under this came the subfamilies Cyprinini, Psilorhynchini and Gobiobotini. In the second group, the gill membrane is free, the suprabranchial organs are present and the pharyngeal bones are perforated. *Hypophthalmichthys* is the only example that I have studied under this subfamily Hypophthalmichthyini.

The osteology of the Cyprinidae has been studied by few workers with a view to establish their relationships. SAGEMEHL (1891) from a comparative study of the skulls was able to divide the family into groups[1], viz., Catostomina, Cyprinina, Leptobarbina, Rasborina, Leuciscina, Rhodeina and Abramadina. STARKS (1926) in describing the ethmoid region of fishes, figured that of two cyprinids, *Labeo* and *Pogonichthys*. KOH (1931) described the osteology of *Carassius* and GREGORY (1933), in his evolution of the fish skull, referred

[1] Omitting Homalopteridae and Cobitidae.

to *Labeo*. SARBAHI (1933) described the skeleton of the Indian cyprinid *Labeo rohita* and GIRGIS (1952) has given an incomplete account of the skull of *L. horie*, employing in certain instances, questionable nomenclature. CHRANILOV (1927) gave a comparative account of the weberian ossicles in a number of cyprinids; similarly, I may refer to the more important descriptions of MATVEIEV (1929), WATSON (1939) and MOOKERJEE, GANGULY and MOOKERJI (1952) on the development and adult structure of the ossicles in various cyprinid examples.

I have examined twelve genera of Gobioninae (and there are, probably, not more than 14—15 genera) and I propose to describe the skull and the weberian apparatus of the subfamily with a view to examine their usefulness in classification. The genera examined are as follows:

1) *Gobio polytaenia*
2) *Gobiobotia pappenheimi* Kreyenberg
3) *Abbottina rivularis* (Basilewsky)
4) *A. psegma* Jordan & Fowler
5) *Saurogobio dumerili* Bleeker
6) *S. dabrayi* Bleeker
7) *Pseudorasbora parva* (Temm. & Schlgl.)
8) *Chilogobio nigripinnis* (Günth.)
9) *C. hainanensis* Nichols & Pope
10) *Hemibarbus maculatus* Bleeker
11) *Sarcocheilichthys variegatus* (Temm. & Schlgl.)
12) *Pseudogobio esocinus* (Temm. & Schlgl.)
13) *Gnathopogon elongatus* (Temm. & Schlgl.)
14) *Leucogobio polytaenia* Nichols
15) *Coricus cetopsis* (Kner)

For purposes of comparison, I have also studied the skull and weberian apparatus of *Catostomus camersonii* (Catostomidae), representing a generalised form of the family.

OBSERVATIONS

The ethmoid region: The supraethmoid (figs. 1—4, se) is a broad median bone which is firmly articulated with the frontals (fr). In forms like *Gnathopogon* (fig. 1), *Gobio*, *Chilogobio*[1], *Sarcocheilichthys*, *Gobiobotia* and *Pseudorasbora* the anteromedian extensions of the supraethmoid are not very prominent; in *Hemibarbus* they are longer than in others metioned. In *Saurogobio*

[1] not considering it as a subgenus under *Sarcocheilichthys* (NICHOLS 1943).

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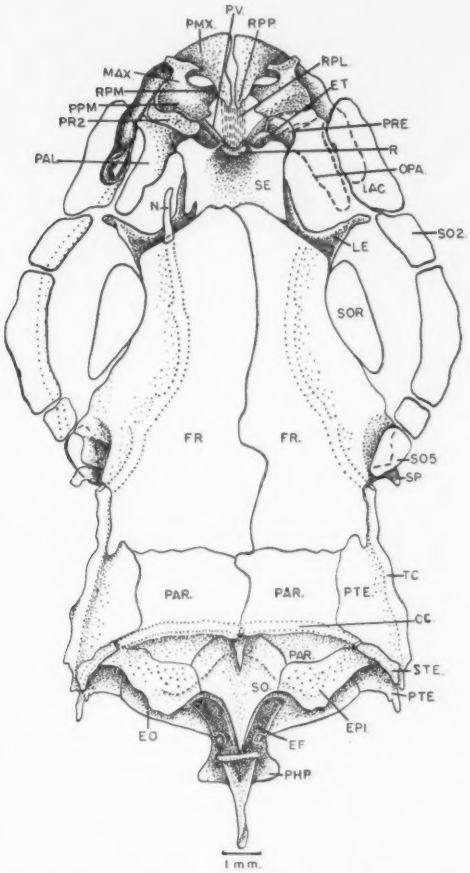
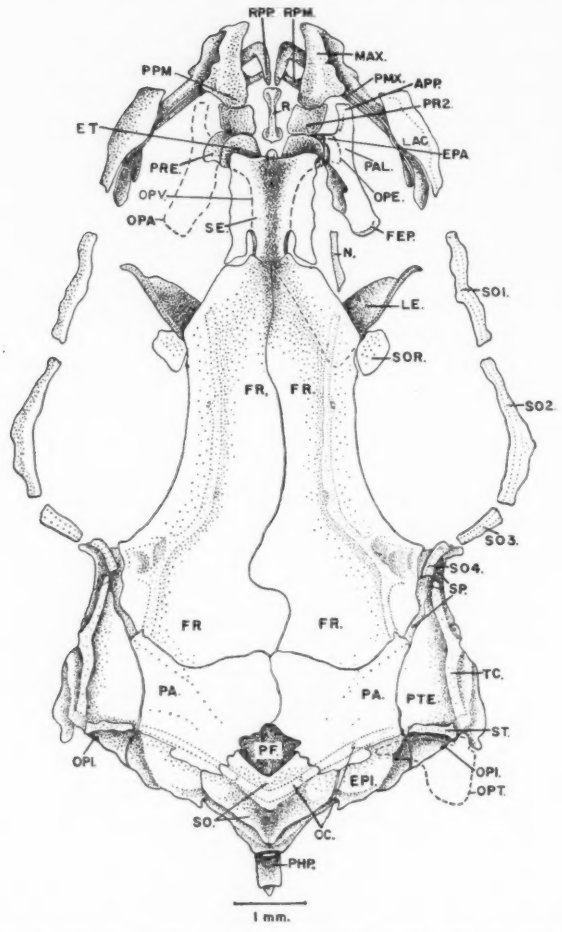


1. Dorsal aspect of the skull of *Gnathopogon elongatus* (Temm. & Schlgl.) (Nasal bone is shown on one side only.)

(fig. 3, aee), *Abbottina* and *Pseudogobio* (fig. 4, aee) prominent anterior extensions are present and in the first two genera, tunnel-like depression in which the median rostral (r) may rest is noticed.

The ethmoid (fig. 1, et) along with the underlying prevomer (pv) gives rise laterally (in front of the supraethmoid) to a conjoint facet covered by cartilage with which a large rounded posterior process of the maxilla (max) articulates. There is usually a cartilaginous meniscus or pad (pr2) between these two articular surfaces. In some examples like *Labeo, Pelecus* and *Rasbora* (SAGEMEHL 1891), this is reduced to a thin fibrous cartilage. However, in *Cyprinus* and *Ablyrhynchichthys* the cartilage undergoes ossification according to Sagemehl; I have also noticed it to be ossified in *Cyprinus*. In Gobioninae, a gradual increase in size of this cartilaginous meniscus takes place in the genera



2. Dorsal aspect of the skull of *Gobiobotia pappenheimi* Kreyen. (Ethmoid region is slightly pulled to show structures clearly; nasal shown on one side only.)

examined by me and I propose to call this intercalated cartilage a second preethmoid (submaxillaria: SAGEMEHL 1891). In *Pseudorasbora*, the second preethmoid is reduced to a thin strut of cartilage; in *Gnathopogon* (fig. 1, pr 2), *Gobio* and *Corieus* the cartilage is more than a strut and in *Gobiobotia* (fig. 2, pr 2), *Chilogobio*, *Sarcocheilichthys* and *Hemibarbus*, it is comparatively longer and in *Abbottina*, *Saurogobio* (figs. 3, 5, pr 2) and *Pseudogobio* (figs. 4, 6, pr 2), it assumes enormous linear proportions, being longest in the last named species. In no genus of the Cyprininae examined by me was the development of the second preethmoid so large as in *Abbottina*, *Saurogobio* and *Pseudogobio*.

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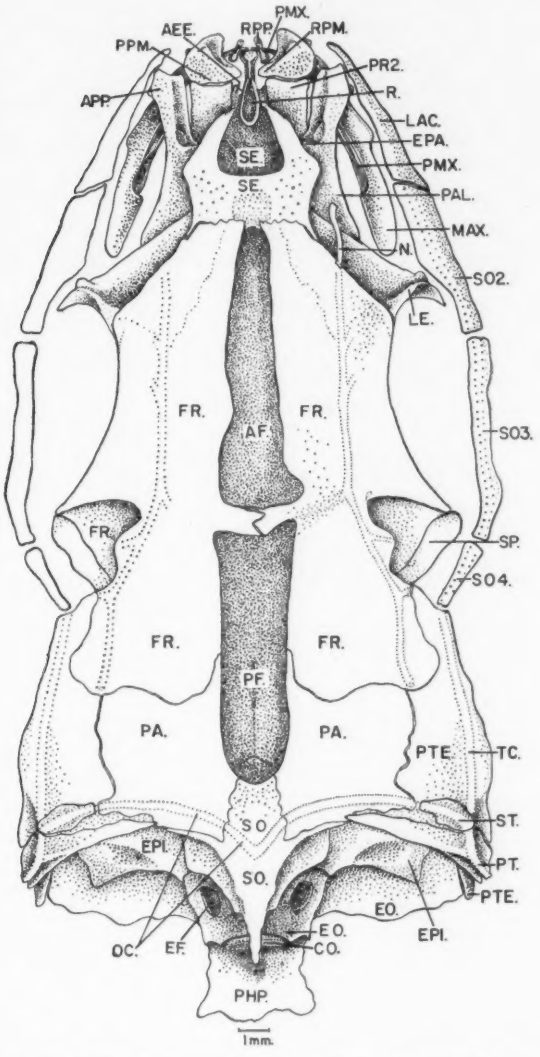
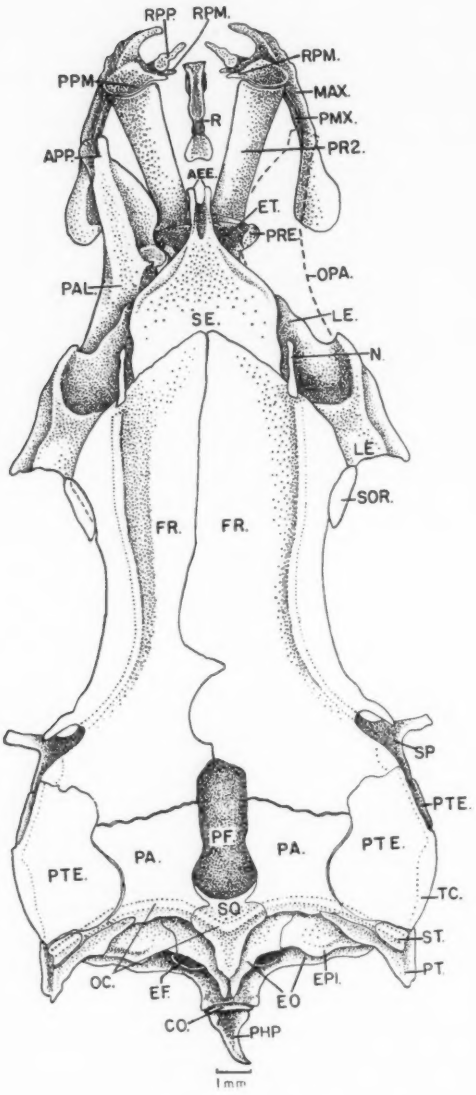


3. Dorsal aspect of the skull of *Saurogobio dumerili* Bleek. (Nasal bone is shown on one side only.)

In the cartilage covering the ethmoid and prevomerine projections referred to above, there is usually an ossification laterally called septomaxillary by SAGEMEHL (1891) but described by later workers (STARKS 1926) as preethmoid (figs. 1, 2, 4—7, pre). In *Abramis, Tinca* and *Chondrostoma* (SAGEMEHL 1891) the preethmoid is absent. I have also noticed the absence of a preethmoid in a few cyprinine genera like *Esomus, Amblypharyngodon, Notropis* etc., and the hypophthalmichthyne *Hypophthalmichthys.*



4. Dorsal aspect of the skull of *Pseudogobio esocinus* (Temm. & Schlgl.) (Suborbital bones are not shown.)

The premaxillae and maxillae show considerable variation. Each premaxilla (figs. 1—6, pmx) shows in the Cyprinidae, a lateral limb and a median process towards the rostral. In *Pseudorasbora* the median rostral process is absent; in *Abbottina*, *Pseudogobio* (figs. 4, 6, rpp) and *Corieus*, just a butt indicates its presence while in *Gobio*, *Saurogobio* (figs. 3, 5, rpp), *Chilogobio* and

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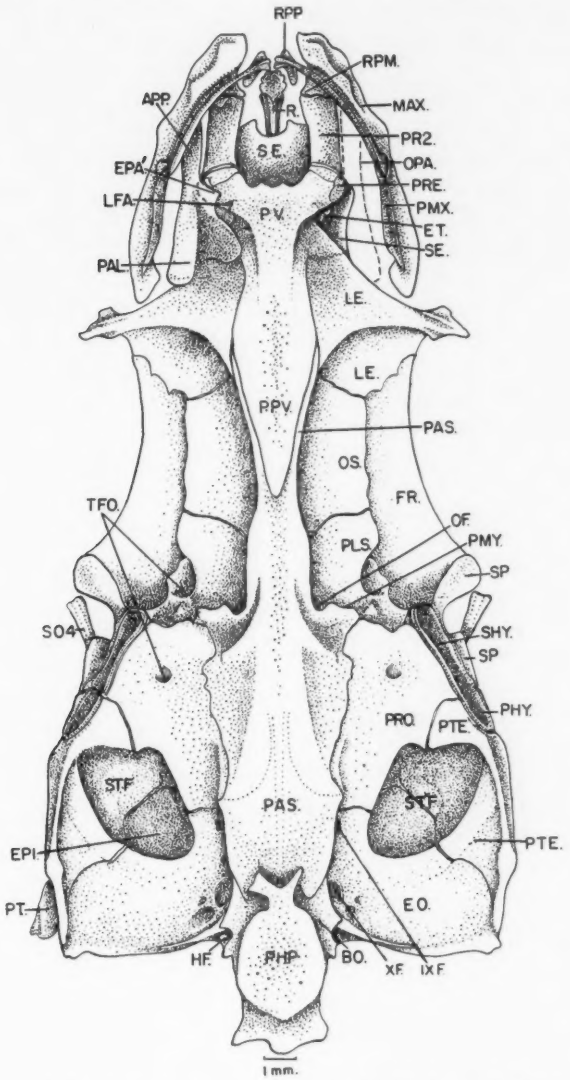
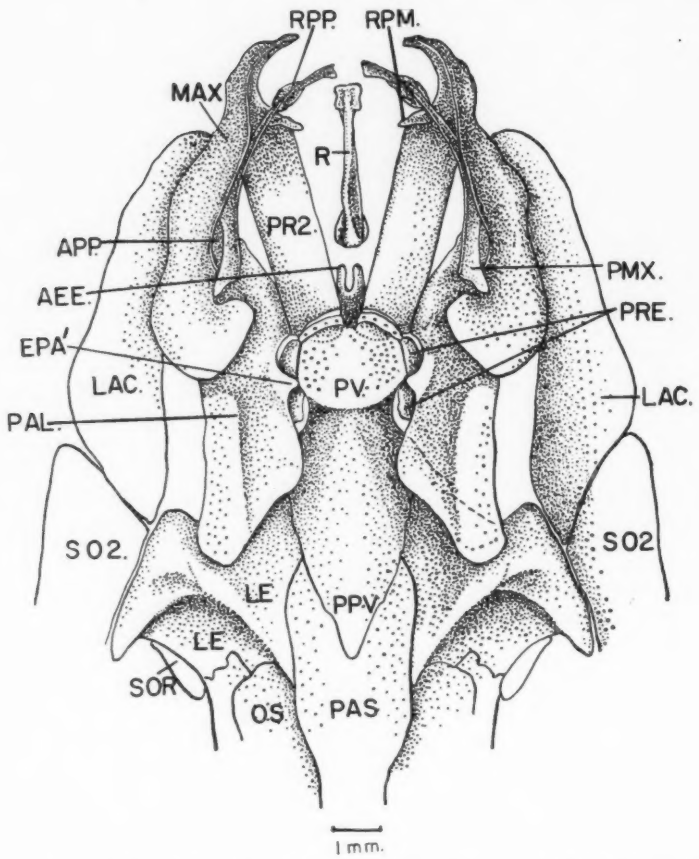


5. Ventral aspect of the skull of *Saurogobio dumerili* Bleek. (Suborbital bones are not shown.)

Sarcocheilichthys a short median process is present. In *Gnathopogon* (fig. 1, rpp), *Gobiobotia* (fig. 2, rpp) and *Hemibarbus* the rostral process is quite large. In *Chilogobio* and *Sarcocheilichthys* the lateral limb of the premaxilla is peculiarly short.

Each maxilla (figs. 1—6, max) shows a mesial and a lateral portion. The mesial portion has a prominent facet (ppm) posteriorly for the second preeth-



6. Ventral aspect of the skull of *Pseudogobio esocinus* (Temm. & Schlgl.), anterior part.

moid (pr 2) and sometimes another rounded facet laterally for the palatine (*Gobio, Pseudorasbora*); it also shows a median rostral process (rpm). The lateral limb may be broad and short as in *Chilogobio, Sarcocheilichthys* and *Pseudorasbora.*

From the rostral processes of the premaxillae, a ligament (fig. 1, rpl) passes over the rostral and is attached to it. The two rostral processes of the maxilla are connected by a ligament and from each one of them, a ligament passes to the median rostral. There is also a ligament connecting the lateral process on the maxilla with the lacrimojugal limb of the palatine.

The median rostral (figs. 1—6, r) element also varies in its development. In *Gnathopogon* (fig. 1), *Chilogobio, Sarcocheilichthys, Gobio, Pseudorasbora* and *Corieus* the rostral is a cylindrical bone obliquely dorsoventrally disposed with the ends enlarged. The dorsal end generally sits upon the anteromedian

portion of the supraethmoid. In *Saurogobio* (figs. 3, 5, r) and *Abbottina* the rostral is also obliquely placed but the dorsal portion shows a large groove for accommodating a median ligament from the premaxillae. In *Pseudogobio* (figs. 4, 6, r) the rostral is linearly disposed in front of the supraethmoid. The rostral also shows considerable variation in the Cyprininae studied by me.

The palatine (figs. 1—6, pal) though belonging to the upper jaw, may be considered here with advantage. In all the genera, the palatine articulates with the lateral aspect of the preethmoid (pre). This process of the palatine is attached to the median projecting portions of the supraethmoid by a ligament. In *Pseudogobio* (fig. 4) this ligament is fairly long since the anterior portion of the supraethmoid is far anteriorly disposed. The palatine shows anterolaterally a short or long process projecting towards the lacrimal; this may be called the lacrimal process. In *Abbottina, Gobiobotia* (fig. 2), *Saurogobio* (figs. 3, 5) and *Pseudogobio* (figs. 4, 6) the lacrimal process (app) of the palatine is fairly long while in *Gobio*, it is just a tiny projection. In *Pseudorasbora* the short lacrimal process is opposite the palatine process of maxilla. Posteriorly the palatine articulates with the anterior end of the entopterygoid in all the genera.

Ventrally the prevomer (figs. 5, 6, pv) forms an important ossification. It extends anteriorly along with the ethmoid (figs. 1, 2, 4, 5, et) in front of the supraethmoid (figs. 1, 2, 7, se) or laterally to it (figs. 5, 6, pv) for the articulation of the palatine (pal) and it has already been noted that the preethmoid (pre) is situated laterally to this projection covered over by cartilage. The prevomer extends posteriorly ventrally to the parasphenoid (pas) and ethmoid bones; a broad posterior limb (*Gobio, Gnathopogon, Chilogobio, Sarcocheilichthys, Abbottina, Saurogobio* (fig. 5, ppv) and *Pseudogobio* (fig. 6, ppv) or short as in *Corieus* (fig. 7, ppv) or short and divided as in *Pseudorasbora* is noticed. In *Hemibarbus* the posterior limb is narrow.

In *Pseudorasbora*, on either side where the prevomer comes in contact with the lateral ethmoid, there is an elevation forming an articular facet (RAMASWAMI, 1953 a) with which an equally prominent facet of the entopterygoid (just posterior to its articulation with the palatine) articulates. This is a new articulation of the upper jaw in fishes. An articulation of the entopterygoid with the *lateral ethmoid* is seen in *Cyprinus carpio* and *Labeo macrostoma* but the prevomer-entopterygoid articulation is not recorded so far.

Orbitotemporal region: The frontals and parietals form the large roofing bones. Generally the bones approximate mesially leaving no fenestra or gaps. In a variety of *C. carpio* (SAGEMEHL, 1891) the occurence of frontoparietal fontanelles has been noted. In a large number of carp skulls examined by me, a small opening in front of the supraoccipital spine has been noticed. In *Gobiobotia* (fig. 2), *Saurogobio* (fig. 3), *Pseudogobio* (fig. 4) and *Abbottina*, fenestrae in the roof are prominent. In *Saurogobio* there is a fontanel between

the supraethmoid (fig. 3, se) and the frontals (fr) and one between the frontals and parietals (pa); in the other genera, there is only one. SAGEMEHL (1891) did not attach importance to the occurence of fontanelles and considered them to be of a fortuitous nature having no physiological significance. TARANETZ (1938) on the other hand, has made use of the presence or absence of an occipital opening for dividing the gudgeons into groups. Probably these bottom dwelling forms need to have these fontanelles for some physiological purpose.

The supraorbital is again a bone showing variation. In *Abbottina, Saurogobio* and *Coricus* the bone is absent while in *Gobiobotia* (fig. 2) and *Pseudogobio* (fig. 4), it is diminutive (sor); in *Gnathopogon* (fig. 1, sor), *Chilogobio, Sarcocheilichthys, Pseudorasbora, Hemibarbus* and *Gobio* it is well formed.

The lateral ethmoid (figs. 1—7, le) (prefrontal of SAGEMEHL, 1891) may be considered as the bone that separates the ethmoid from the orbitotemporal region. The bone does not give rise to a lacrimal process as in Homalopteridae (RAMASWAMI, 1948, 1952 c). In *Coricus* the lateral ethmoid (fig. 7, le) shows ventroposteriorly a prominent facet (fea) with which a facet of the entopterygoid articulates. Similar facets are also noticed on the lateral ethmoid of *Cyprinus* and the African species, *Labeo macrostoma*. In *Labeo rohita* and other species of *Labeo* (*horae, niloticus*), I have not noticed this articulation. GIRGIS (1952) also does not record a facet on the entopterygoid in *L. horae* whose skeleton he described.

The number of suborbital bones varies. The first one (figs. 1—3, 6, 7, lac) is usually called the lacrimal. Five seems to be the common number (including the lacrimal) and in *Abbottina*, one side has only four[1]; *Saurogobio dumerili* (fig. 3, so 2—so 4) has only four ossicles while *dabrayi* has five ossicles.

It is interesting to note that in some examples like *Cyprinus* and *Carassius* the two orbitosphenoids unite together to form a single bone so that between them, no suture is visible dorsally. However, they do give rise ventrally to a median portion with which a lamina of the parasphenoid comes in contact to form the interorbital septum. In the majority of the cyprinids examined by me at the British Museum (Natural History), London, at the Natural History Museum, Stanford University, Calif., and at the department of zoology, University of Wisconsin, Madison, I noticed that when the frontals are taken away and the tissue cleared, the orbitosphenoids are noticed as paired bones. I have guardedly stated that this feature occurs in the majority of the examined genera of cyprinids since I did not remove the frontals from the dry-skull museum exhibits. In *Catostomus*, the orbitosphenoids are double; in all the Cobitidae (RAMASWAMI 1953) the orbitosphenoid is a united bone and in fact, this is one of the distinguishing characters of the family.

The pleurosphenoid (figs. 5, 7, pls) (alisphenoid of literature) is the bone that forms the posterior boundary of the orbit and usually the pleurosphenoid,

[1] I have examined more than four specimens.

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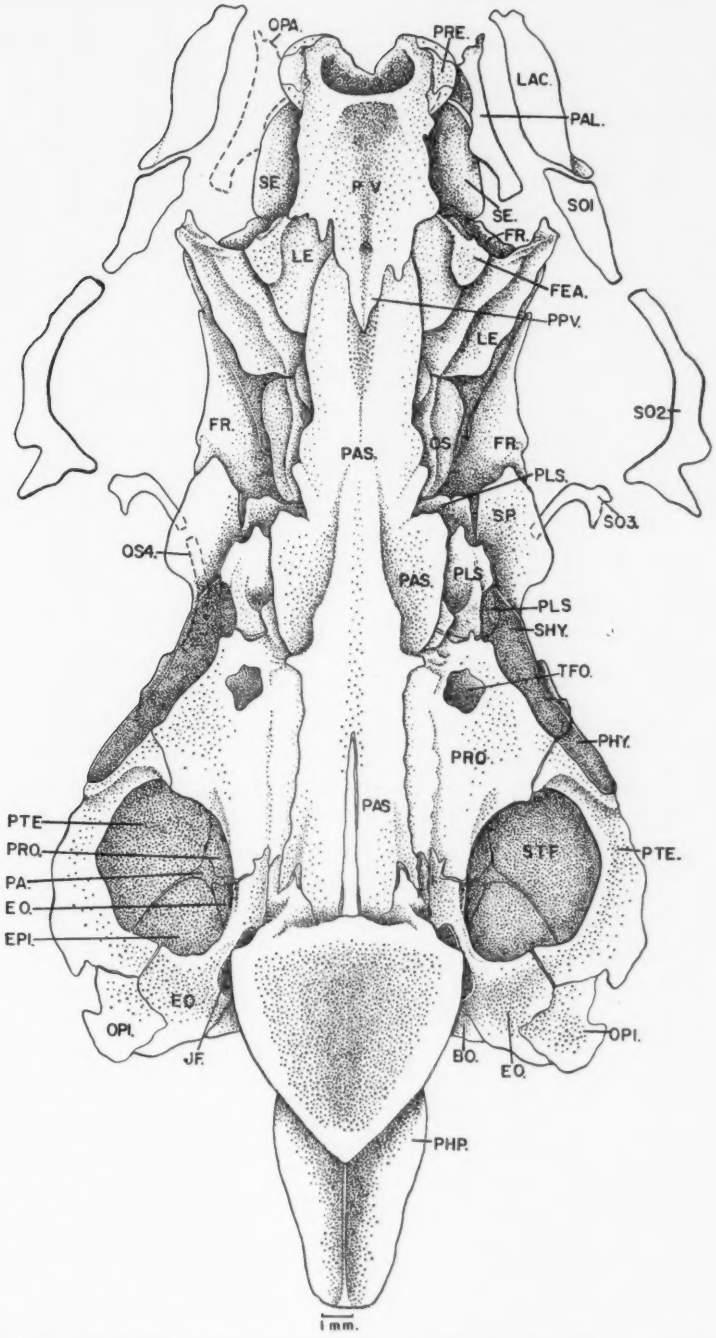

7. Ventral aspect of the skull of *Coreius cetopsis* (Kner). (Maxillae, premaxillae and rostral are not included.)

parasphenoid, and the orbitosphenoid bound the optic foramen. The large pleurosphenoid may also bound the hyomandibular facet (figs. 5, 7, shy) anteriorly along with the sphenotic (sp) as in *Corieus, Gobio, Gnathopogon, Hemibarbus, Suarogobio, Pseudorasbora, Chilogobio* and *Sarcocheilichthys* and in a few cases, the pleurosphenoid is kept out by the prootic and the sphenotic (*Gobiobotia, Abbottina* and *Pseudogobio*).

In the African species *Labeo macrostoma* examined by me, the pleurosphenoid bounds the hyomandibular facet anteriorly. In *L. rohita* (SARBAHI 1933) and *L. niloticus*, the same condition is met with while in *L. horie*, GIRGIS (1952) does not throw any light on this point. SAGEMEHL (1891) however, pointed out that *Labeo* was an exception where the pleurosphenoids did not bound the articular facet.

The parasphenoid (figs. 5, 6, 7, pas) forms the median floor not only in the occipital region but also in the orbitotemporal and ethmoid regions. Except that it may be very broad posteriorly as in *Saurogobio, Abbottina* and *Pseudogobio*, not much variation is noticed in the structure of the bone.

The auditory region: This region is bounded by the frontals, parietals, sphenotics, pterotics, epiotics, sometimes opisthotics, supratemporals and supraoccipital. The sphenotic (figs. 1—5, 7, sp) situated laterally to the pleurosphenoid and prootic and anteriorly to the pterotic forms a partial anterolateral boundary for the hyomandibular facet; the bone also shows a short projection anterolaterally to the hyomandibular facet in all the examined genera. On the dorsal aspect of the skull, the pterotic and frontal meet together and only the projection of the sphenotic (referred to above) is seen; however, in *Gobiobotia* (fig. 2), the pterotic (pte) and frontal (fr) leave a gap between them in which the sphenotic is visible.

The pterotic (figs. 1—5, 7, pte) bounds laterally the subtemporal fossa (figs. 5, 7, stf) and exhibits a facet (phy) for the hyomandibular articulation. Dorsally, the temporal sensory canal (figs. 1—3, tc) runs in the bone, which is a continuation from the frontal. From the anterior end of the pterotic, the sensory canal also branches off to the suborbital series to end in the snout on the lacrimal bone.

Between the exoccipital and the posterior portion of the pterotic, a large opisthotic is noticed in *Corieus* (fig. 7, opi); in *Gobiobotia* (fig. 2, opi) there is a tiny ossicle representing the opisthotic.

The prootic (figs. 5, 7, pro) forming the anteromesial boundary of the subtemporal fossa (stf) shows usually two orifices (tfo) for the passage of fifth and seventh cranial nerve branches.

The epiotic (figs. 1—5, 7, epi) (occipitale externum: SAGEMEHL 1891) forms the posterior boundary and also the roof of the subtemporal fossa. It generally shows a small process posteriorly for the attachment of a ligament.

Connecting the temporal canal (figs. 1—4, tc) on the pterotic (pte) and the

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occipital canal, there is a small ossicle, — the supratemporal (st). The post-temporal leads the sensory canal into the lateral line; its mesial limb comes in contact with the epiotic and in *Saurogobio* (fig. 3), it extends as far as the parietal.

It is interesting to note that in *Gnathopogon* (fig. 1), the occipital canal (oc) runs in the parietals; this is also the condition in the Cyprininae generally. In *Gobiobotia* (fig. 2), *Saurogobio* (fig. 3), *Pseudogobio* (fig. 4) and *Abbottina*, the occipital canal also passes through the supraoccipital. In *Catostomus* the sensory canal ossicles are separate from the roofing bones.

The supraoccipital (figs. 1—4, so) roofs the occipital region and generally there is a supraoccipital process posteriorly. The foramen magnum is completed dorsally by the two exoccipitals (eo) (occipitale laterale: SAGEMEHL 1891) and the supraoccipital is kept out. The two exoccipitals are large ossifications and show the characteristic lateral fenestrae (ef) and orifices for the ninth (fig. 5, IXf) and tenth (Xf) cranial nerves and spinal nerves (hf). Sometimes there may be a single foramen for the exit of glossopharyngeal and vagus nerves (fig. 7, jf). The exoccipitals bound the subtemporal fossae (stf) posteriorly. The basioccipital exhibits the large pharyngeal process (php) and the median concave condyle. Dorsally to the pharyngeal process, a passage for the artery could be clearly made out.

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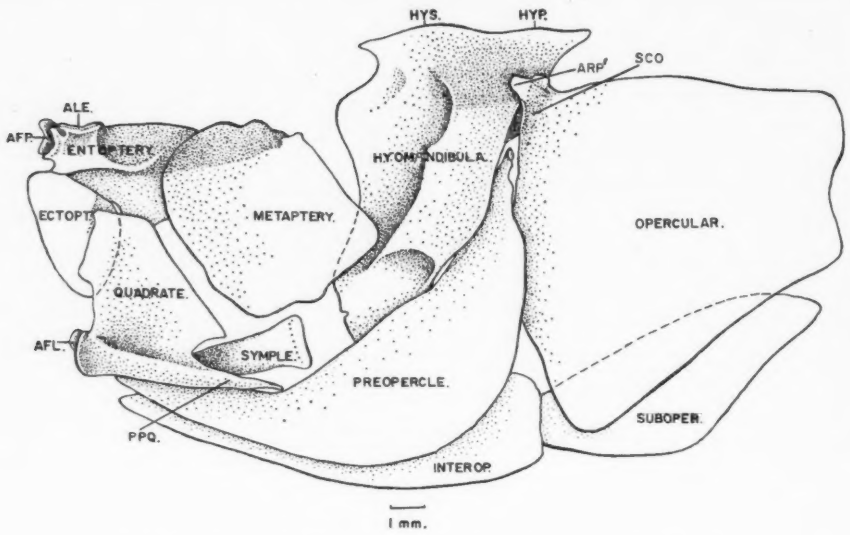
The upper and lower jaws: The upper jaw of the fish articulates with the cranium anteriorly by its palatine and posteriorly by the hyomandibula. The palatine has a mesial process (fig. 3, 5, epa) which is attached to the anterior supraethmoid process by a ligament; by another articular facet (figs. 5, 6, epa'), the palatine articulates with the preethmoid laterally. Posteriorly it is connected with the entopterygoid (fig. 8, afp). The entopterygoid usually does not have any articular facets other than the ones with the ecto- and metapterygoids and anteriorly with the palatine. However, in the African *Labeo macrostoma* and in *Cyprinus carpio*, the entopterygoid develops a facet mesially to articulate with a similar facet on the lateral ethmoid. I have also noticed a similar facet in *Corieus* (fig. 8, ale). In *Pseudorasbora* the entopterygoid articulates by a prominent facet with one on the prevomer, anterior to the lateral ethmoid bone (RAMASWAMI, 1953 a). It is not clear why in the free-swimming carp or in the bottom dwelling gudgeon, additional attachments should be developed in the upper jaw. The metapterygoid does not show any variation. The ectopterygoid (fig. 8) situated in front of the quadrate, assumes larger proportions in forms like *Saurogobio* and *Corieus*; in *Abbottina* the entopterygoid and ectopterygoid are fused. The hyomandibula articulates dorsally with the sphenotic-pterotic bones. It may have a single head for articulation as in *Labeo rohita* (SARBAHI 1933) or, as in the majority of fishes by two heads (hys, hyp). By a prominent head posteriorly, it gives articulation to the opercular. The symplectic may be a fairly long slender bone or it may



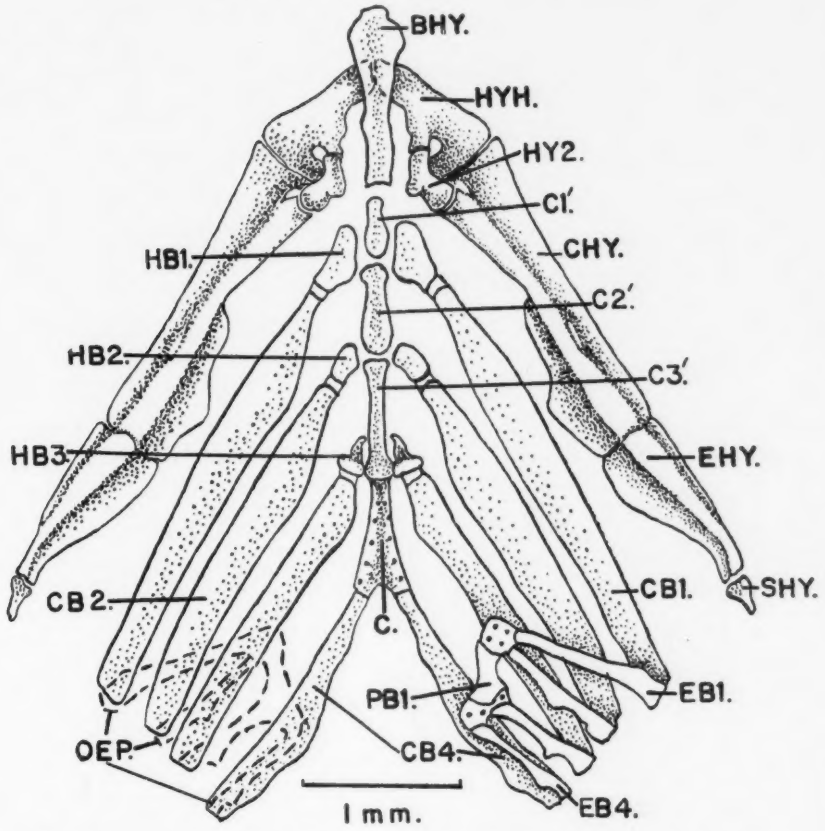
8. Upper jaw and Opercular bones of *Coricus cetopsis* (Kner).

be short. This bone is a constant feature of the upper jaw of most fishes. The quadrate articulates with the ectopterygoid dorsally and with the anterior portion of the symplectic by the posterior membranous extension, — the posterior process of the quadrate (fig. 8, ppq). Peculiarly in *Sarcocheilichthys*, the posterior process of the quadrate is very short.

The opercular series consists of opercular (fig. 8), preopercular, interopercular (interop) and subopercular (suboper). The opercular is the largest of the series and usually it shows an articular process (arp) towards the hyomandibula. In a few examples (*Chilogobio, Sarcocheilichthys, Pseudogobio, Gnathopogon, Hemibarbus, Saurogobio, Gobiobotia* and *Coricus*) the opercular carries the sensory canal (sco) leading to the lower jaw; in *Abbottina* and *Pseudorasbora* a canal is absent in the opercular. In *Pseudogobio* the opercular is large and the subopercular is reduced to a small triangular bone between the opercular and interopercular. In *Saurogobio*, the interopercular sends up a fairly long limb towards the preopercular. The subopercular of *Abbottina* has mesially a socket into which a boss of the interopercular fits in.

In the lower jaw, the following characteristic bones are met with: edentulous dentary, angular, retroarticular and sesamoid angular. The dentary carries the sensory canal laterally in it and only in *Pseudorasbora*, there is a posteriorly directed spinous process from the dentary.

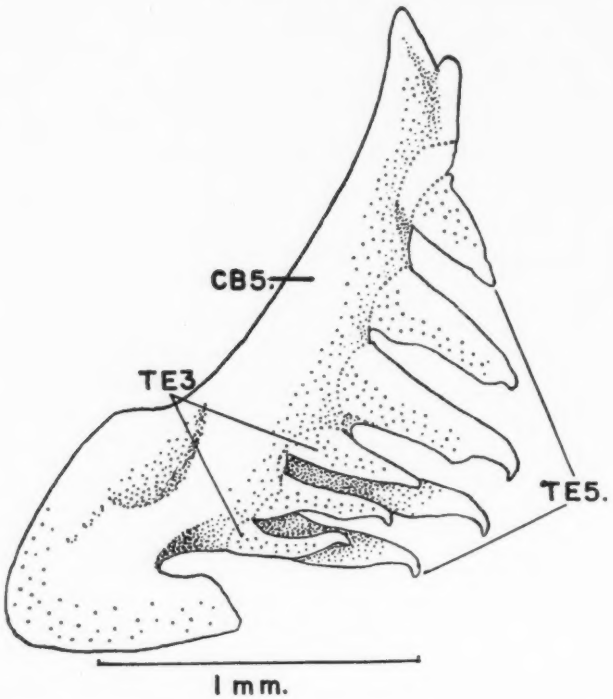
The hyobranchial apparatus: The cyprinid hyobranchial apparatus does not show much variation. The median elements are the basihyal (fig. 9, bhy) (sometimes called glossohyal) and three copulae (c1′, c2′, c3′) followed by a



9. Dorsal aspect of the hyobranchial apparatus of *Gobiobotia pappenheimi* Kreyen.

strut of cartilage. These copulae represent fused basibranchials. Sometimes a calcification posterior to the third copula may also be noticed as in *Pseudogobio*. There are usually two pairs of hypohyals (fig. 9, hyh, hy2) (sometimes labeled as basihyal and hypohyal, when the median piece of bone is called entoglossum or glossohyal); a pair each of ceratohyals (chy) and epihyals (ehy) is also noticed. A small stylohyal (shy') connects the hyoid cornu with the hyosymplectic. The segments of the hyoid cornu may be abbreviated in size as in *Sarcocheilichthys, Chilogobio, Gnathopogon* and *Leucogobio*. Between the ventral pair of hypohyals, there is an I-shaped ossicle around which the hypohyals can rotate in *Abbottina, Saurogobio, Sarcocheilichthys, Pseudorasbora* and *Pseudogobio*.

In the branchial arches, there are three pairs of hypobranchs (fig. 9, hbl—hb3), of which the third pair shows a projection ventrally. In *Abbottina, Saurogobio* and *Pseudogobio* the hypobranchs are longer than in the other



10 a. Left inferior pharyngeal bone of *Gobiobotia pappenheimi* Kreyen.

genera examined. In *Abbottina* peculiarly the first pair of hypobranchs articulate with the hypohyals. The ceratobranchs (cbl—cb4) are five pairs in number and in the first four pairs of branchial arches, they are followed by the epibranchs (ebl—eb4); the fifth pair is the pharyngeal bone showing the characteristic dentition. The terminal extremities of the epibranchs still retain cartilaginous connexion, in which two ossifications, viz., the edentulous pharyngobranchs are noticed. These probably represent the fused first and second, and the third and fourth pharyngobranchs. In *Gobiobotia* I notice only one pair of pharyngobranchs (fig. 9, pbl). In the fifth arch (figs. 10 a, 10 b, cb 5), there is only the ceratobranch also called the inferior pharyngeal bone, carrying teeth. In describing the branchial arches of *Labeo*, GIRGIS (1952) referred to these bones as the fifth pair of epibranchs, the ceratobranchs themselves being represented by 'threadlike unpaired cartilage'. This homology of the segments of the fifth arch is erroneous, for in all the descriptions of the hyobranchial apparatus, only one segment is noted and that corresponds with the ceratobranch.

The pharyngeal teeth show considerable variation both in the nature of

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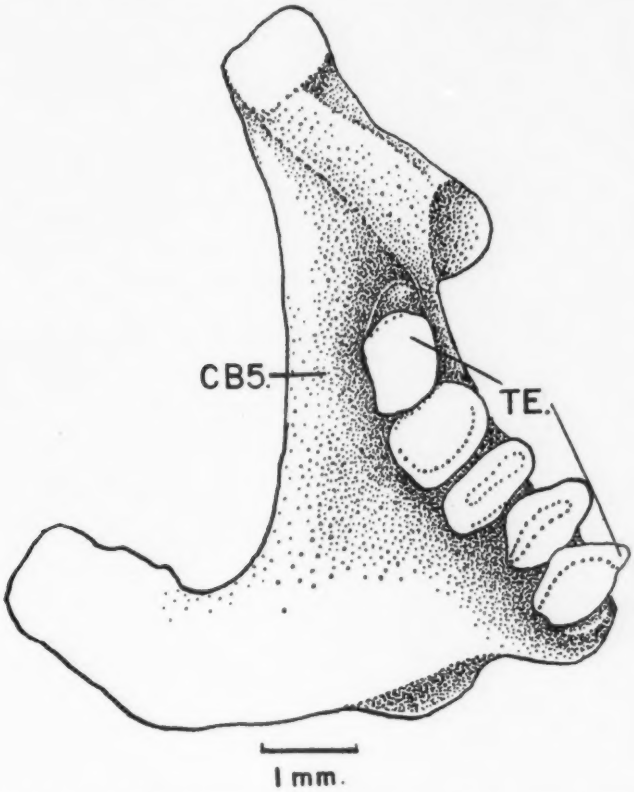
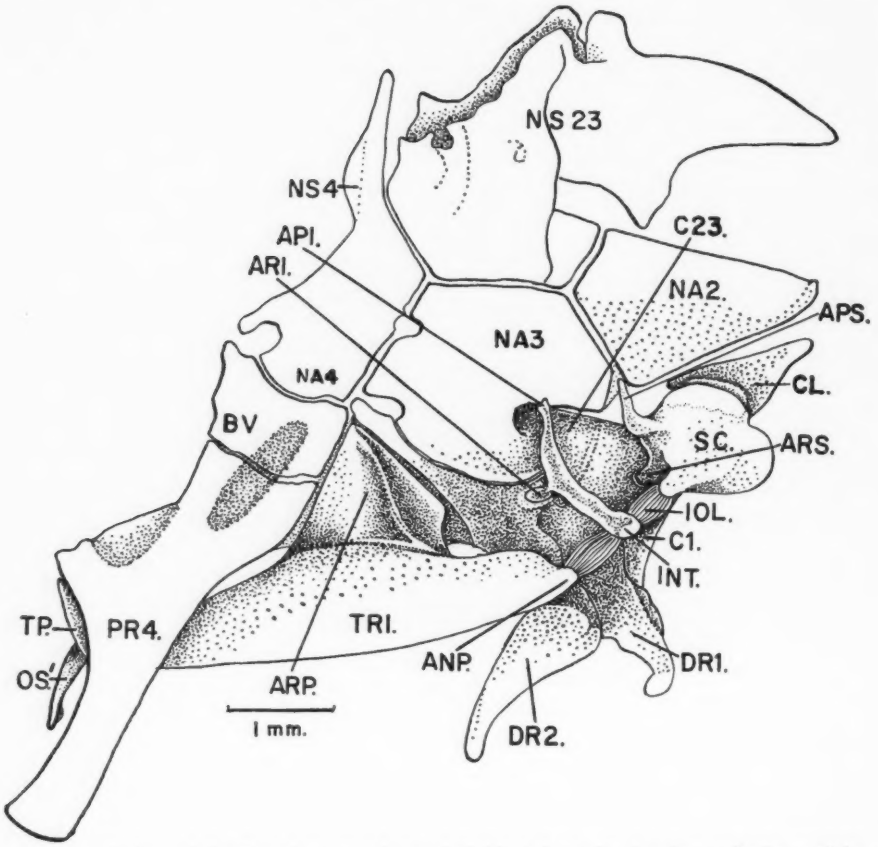


10 b. Left inferior pharyngeal bone of *Saurogobio dumerili* Bleek.

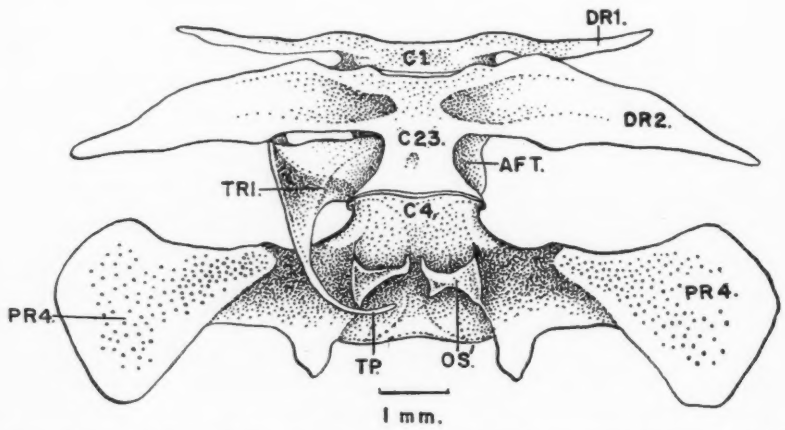
teeth and their number. Listed below are the pharyngeal dental formula and the rows they form in the Gobioninae:

	According to Chu (1935): dentition:	rows:	According to author: dentition:	rows:
Hemibarbus	1, 3, 5—5, 3, 1	3	1, 3, 5—5, 3, 1	3
Gnathopogon	2 or 3, 5—5, 3 or 2	2	2, 5—5, 2	2
Gobio	3, 5—5, 3	2	3, 5—5, 3	2
Pseudogobio	1 or 2, 5 or 6—6 or 5, 2 or 1	2	2, 5—5, 2	2
Chilogobio	0 or 1, 5—5, 1 or 0	1 or 2	1, 5—5, 1	2
Sarcocheilichthys	0 or 1, 5—5, 1 or 0	1 or 2	5—5	1
Gobiobotia	—	—	3, 5—5, 3	2
Pseudorasbora	5—5	1	5—5	1
Abbottina	4 or 5—5 or 4	1	5—5	1
Saurogobio	5—5	1	5—5	1
Corieus	5—5	1	5—5	1

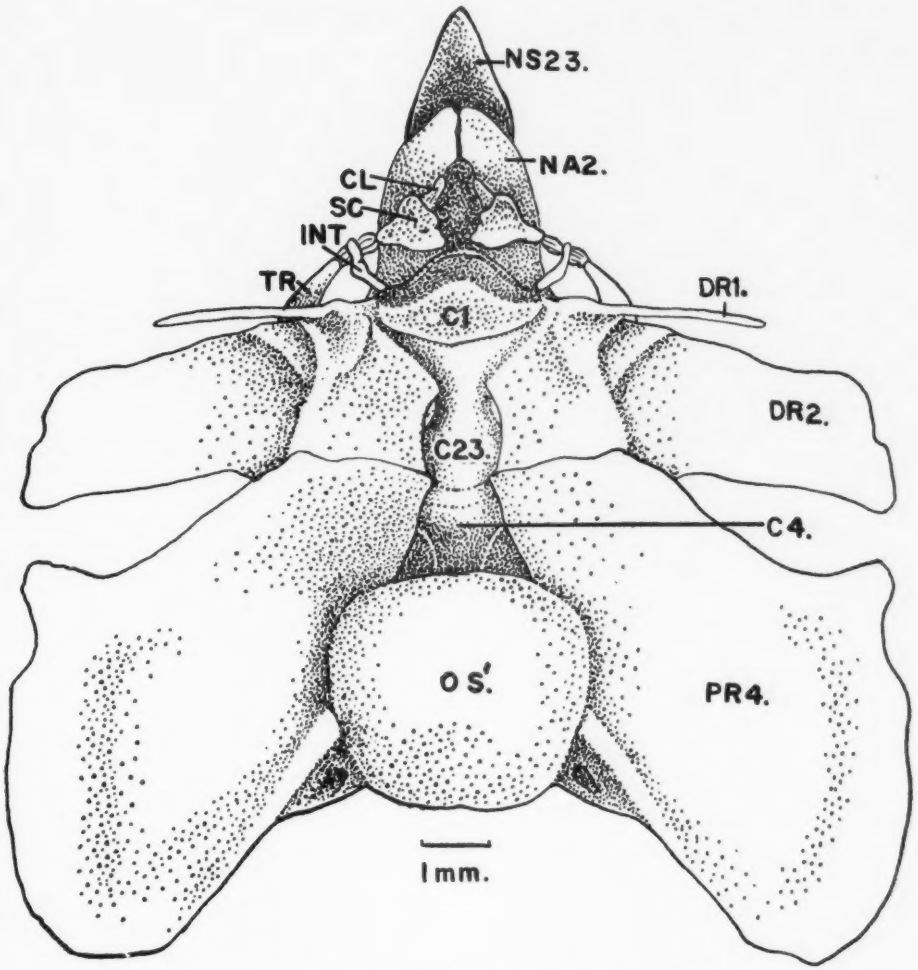
The number of teeth rows is generally taken as an indication of the primitiveness of the animal; three rows are considered as the most primitive type



11. Lateral view of the first four vertebrae of *Gobio polytaenia*, showing weberian ossicles.



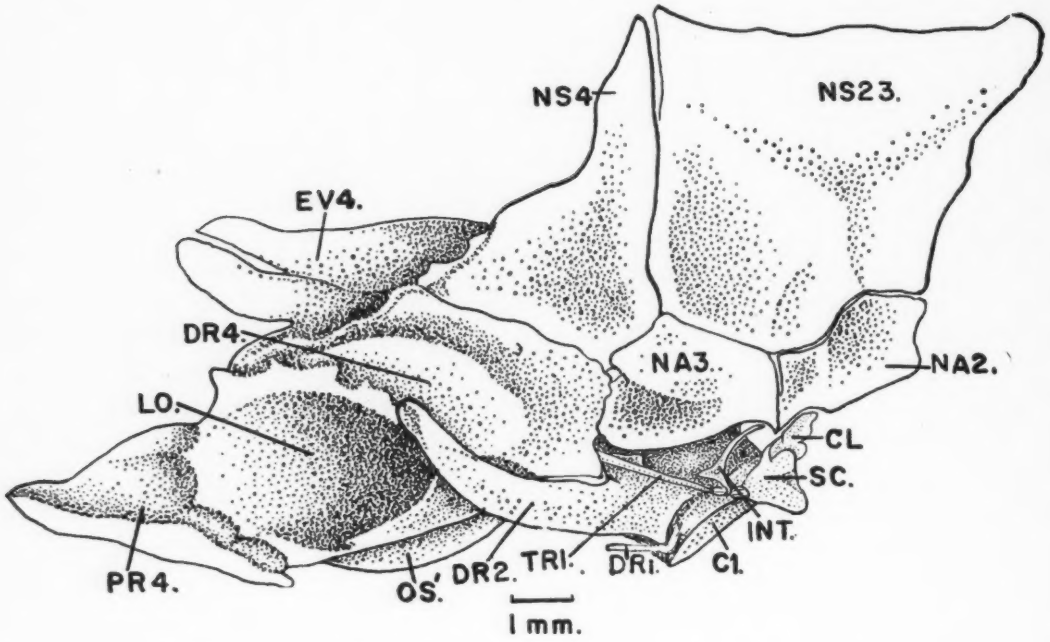
12. Ventral view of the first four vertebrae of *Pseudogobio esocinus* (Temm. & Schlgl.)



13. Front view of the first four vertebrae and gasbladder capsule of *S. dumerili* Bleek.

(REGAN, 1908; CHU, 1935; VASNECOV, 1939; NICHOLS 1943) but BERG (1912) is inclined to think that possessing a row of teeth is a feature of primitiveness. Assuming that greater number of rows mean primitiveness, *Hemibarbus* would be the most primitive genus and then come those with two rows, viz., *Gnathopogon, Chilogobio, Sarcocheilichthys, Gobio* and *Gobiobotia* (fig. 10 a). *Saurogobio* (fig. 10 b), *Abbottina, Pseudorasbora* and *Corieus* are more recent forms. But the two former genera, in showing a number of catostomid characters, are probably more primitive than others.

The weberian apparatus and gasbladder capsule: The first four vertebrae

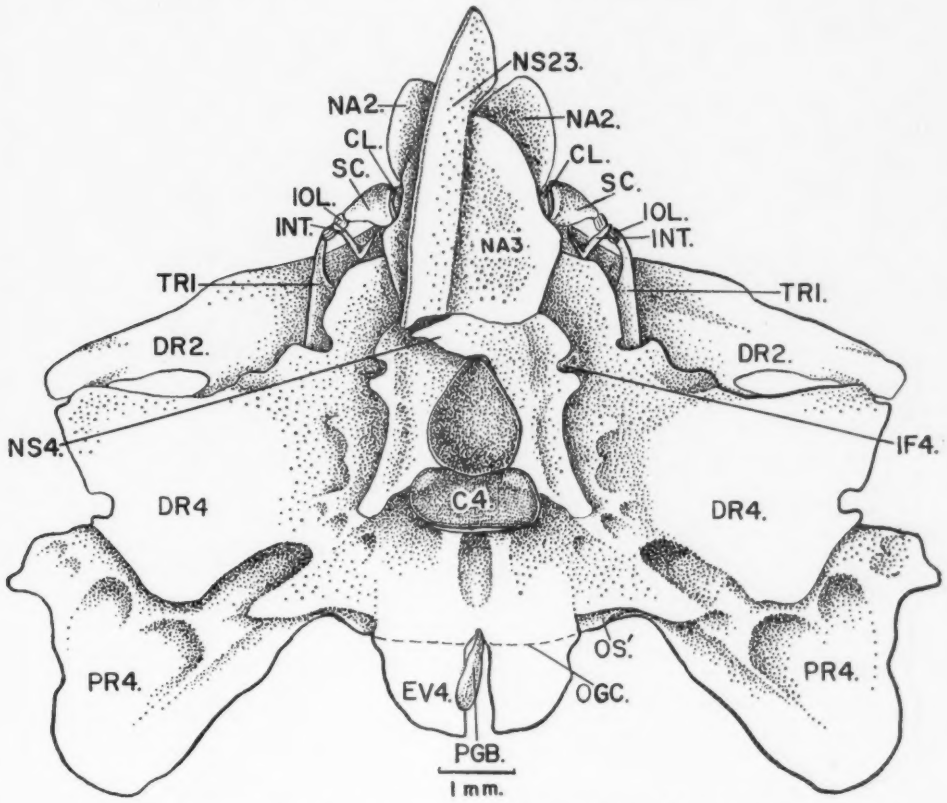


14 a. Lateral view of the first four vertebrae and gasbladder capsule of *Saurogobio dabrayi* Bleek.

contribute towards the formation of the weberian apparatus and the gasbladder capsule in the Ostariophysi. When the gasbladder is encased as in the Homalopteridae (RAMASWAMI, 1952 c), Gastromyzonidae (RAMASWAMI, 1952 d) and Cobitidae (RAMASWAMI 1953) and some Gobioninae (*Saurogobio, Gobiobotia*), the lateral parts of it are usually without a bony wall where the thin skin of the animal comes in contact with the gasbladder. In *Saurogobio* there is a ventromedian rounded portion of the capsule (fig. 13, os') also; from a median orifice in this, the short posterior portion of the gasbladder (fig. 14 b, pgb) projects. Such a part of the capsule is not seen in *Gobiobotia* (fig. 15); this resembles more the homalopterid or cobitid type.

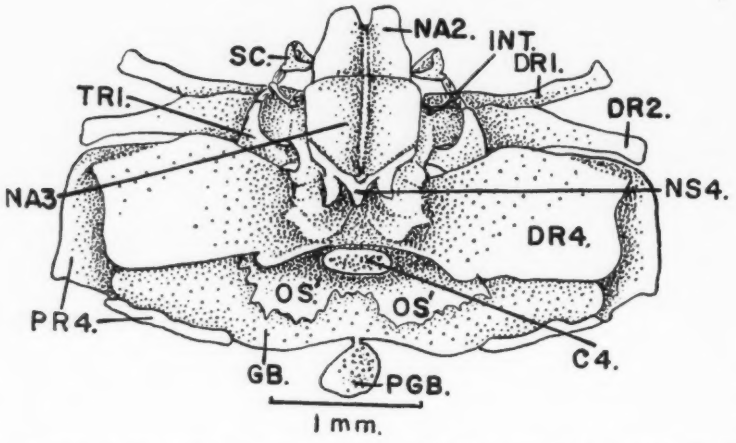
The weberian apparatus is usually described under two heads: a) pars sustentaculum consisting of vertebral parts, and b) pars auditum consisting of the ossicles.

In the Gobioninae, the first vertebra (figs. 11—14 b) has a small centrum (c'1) whose anterior and posterior facets are flat. In some Cyprininae (*Esomus, Notropis, Opsariichthys*) the second and third centra are not fused and the first centrum shows posteriorly a concavity. A pair of dorsal ribs (dr1) arises from the first centrum. The second centrum is usually united with the third (figs. 12, 13, c23); in some Cyprininae, as stated above, they may also be



14 b. Dorsal view of the first four vertebrae of *S. dabrayi* Bleek.

disunited. In the Gobioninae a pair of large dorsal ribs (figs. 11—15, dr 2) and a neural spine (probably united with the third, ns 23) are associated with the second vertebra; in *Saurogobio* and *Gobiobotia* where a capsule is formed, the dorsal ribs (dr 2) are broadened out, but do not take part in the formation of the anterior capsular wall as they do in Cobitidae (RAMASWAMI 1953). The third centrum in Gobioninae is united with the second and where it is free, it is amphicoelous. The third vertebra shows a large neural arch (figs. 11, 14 a, 14 b, 15, na 3) with a large neural spine probably united with the second (ns 23), which latter extends over the first and second vertebrae (fig. 11). No other processes are noticed from the third vertebral centrum. The fourth vertebra exhibits an amphicoelous centrum, a well developed neural arch and spine (ns 4); in the Gobioninae without a gasbladder capsule, the pleural ribs arising from the fourth centrum broaden out (fig. 12, pr 4). In *Gobio*, the pleural rib (fig. 11, pr 4) arises from a portion on the side of the centrum which probably represents a parapophysis (basiventral). In *Saurogobio* and



15. Dorsal view of the first four vertebrae and gasbladder capsule of *Gobiobotia pappenheimi* Kreyen.

Gobiobotia the dorsal ribs of the fourth vertebra (figs. 14 a, 14 b, 15, dr 4) are broadened out to form the roof of the capsule while the broadened pleural ribs (figs. 13, 14 a, 14 b, 15, pr 4) form the ventral portion; the ossa suspensoria (figs. 13, 14 a, 14 b, os') appear to form the median portion of the capsule in *Saurogobio* while in *Gobiobotia* (fig. 15) it appears to form just a median portion of the roof for the anterior gasbladder (gb).

Of the pars auditum, claustrun (figs. 11, 13, 14 a, 14 b, cl) is the first ossicle and it hangs from the side of the second neural arch (na 2). With regard to its origin, WATSON (1935) noted in the goldfish that it arose as an independent chondrification and therefore, did not represent any vertebral part. The next ossicle is the cupshaped scaphium, the claustrum forming a lid for the cup. The scaphium is connected with the tripus by the interossicular ligament. It shows articular (fig. 11, ars) and ascending (aps) processes. In the goldfish, these latter two processes are derived from the basidorsal cartilage of the first vertebra, the concha being of membranous origin. The intercalarium (figs. 11, 13, 14, 15, int) is the third ossicle also showing ascending (api) and articular (ari) processes. Watson derived these processes from the basidorsal of the second vertebra while the manubrium incudis, imbedded in the interossicular ligament was of ligamentary origin in the goldfish. The largest of the ossicles is the fourth one, viz., the tripus (fig. 11, tri) connected by the interossicular ligament (iol) with the scaphium (sc). The tripus articulates with the lateral aspect of the centrum of the third vertebra by its articular process (arp) and with the gasbladder posteriorly by its transformator process (figs. 11, 12, tp). In the goldfish, the tripus is noticed to arise partly in membrane, partly from the basiventral cartilage of the third vertebra and

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partly from its rib. The transformator process is present in all the genera studied except *Gobiobotia* and *Saurogobio* where the gasbladder is enclosed in a capsule.

TARANETZ (1937) who studied the gasbladder capsule of *Saurogobio dabrayi* and *dumerili*, thought that they differed very much and therefore, he raised the latter species to the rank of a new genus, *Armatogobio*. I, however, do not consider the differences so important as to split them into different genera.

DISCUSSION

That the members of the subfamily Gobioninae do not form a natural assemblage is clear from the way systematists have split the group from time to time.

MORI (1934) considered the members of Gobioninae as belonging to two subfamilies, Gobiobotinae (*Gobiobotia, Saurogobio*) and Cyprininae; the latter having groups or tribes called Barbini (*Hemibarbus*) and Gobioninae (*Gobio, Pseudogobio, Pseudorasbora, Gnathopogon*). TARANETZ (1938) after studying the gudgeons of the Amur river concluded that the inferior mouth of these forms was derived from the terminal mouth of a least specialised form like *Gnathopogon* by two methods and divided accordingly the members under two heads. NICHOLS (1943) however, included the Gobioninae under Cyprininae.

A study of the skeletal structures appears to throw light on the interrelationship of these genera; in the subfamily Gobioninae, *Saurogobio, Pseudogobio* and *Abbottina* fall into a group while the rest, for the sake of convenience, may be considered to fall into another. However, *Gobiobotia* shows a number of specializations.

In the ethmoid region, the three genera (*Saurogobio, Pseudogobio* and *Abbottina*) show elongated second preethmoid cartilages spanning between the rostral process of the maxilla and the prevomerine projection. *Catostomus*[1] (Catostomidae) also shows such elongated cartilages. In the other examples of Gobioninae, this cartilage is very much smaller, and in a few Cyprininae, this tiny cartilage even becomes ossified.

In Cyprininae and *Catostomus*, each premaxilla shows a process towards the median rostral called the median rostral process. In *Saurogobio, Pseudogobio* and *Abbottina* it may be absent or represented as a small projection; in *Gobiobotia*, the median rostral process of premaxilla is well developed.

The median rostral is elongated in *Saurogobia, Abbottina, Pseudogobio* and *Gobiobotia* and in the former two genera, the posterior portion sits in a groove of the sphenethmoid.

In *Saurogobio, Abbottina* and *Pseudogobio* the prevomer has a prominent

[1] I have examined only this genus.

posterior process; and in the ventral view, the anterior portion of the prevomer shows a sharp ventral bend, also seen in *Catostomus*. In *Gobiobotia* the prevomer has a prominent posterior process but no flexure is seen.

In the Cyprininae generally and in *Catostomus*, the lateral ethmoid is well developed with a lateral process towards the lacrimal called the lacrimojugal process. In *Saurogobio*, *Abbottina*, *Pseudogobio* and *Gobiobotia* the lateral process is absent.

In *Gobiobotia*, *Abbottina*, *Saurogobio* and *Pseudogobio*, the palatine sends anteriorly a lacrimal process which is longer than in other genera examined; in *Catostomus* the lacrimal process is short.

There are usually one or two fontanelles in the frontal-parietal region; in *Saurogobio* the anterior one is between the frontals and the posterior one between frontals and parietals. In *Pseudogobio* and *Abbottina* there is only the posterior one. In *Gobiobotia* the fontanel is between the supraoccipital and the parietals; in the other Gobioninae there is no fontanel. In *Catostomus*, a fontanel is present between supraoccipital, frontals and parietals. In *Cyprinus* there is a small fontanel in the supraoccipital.

The orbitosphenoids are double in the Gobioninae; this is also the case in *Catostomus* and majority of Cyprininae. Like the Cobitidae, the orbitosphenoid is a united bone in *Cyprinus* and *Carassius*.

The supraorbital may be present or absent; in *Saurogobio*, *Abbottina* and *Coricus* it is absent while in *Gobiobotia* and *Pseudogobio*, it is diminutive. In *Catostomus* also it is absent.

There is one other singular feature in which *Gobiobotia*, *Saurogobio*, *Pseudogobio* and *Abbottina* stand apart from the other members of Gobioninae examined. The occipital canal passes through the parietals without entering the supraoccipital generally but in the above genera, it passes through the supraoccipital also. This would be one of the very important points to include *Gobiobotia* in Gobioninae as it stands today.

In possessing a bony covering for the gasbladder, *Saurogobio* and *Gobiobotia* stand apart from the other members of the Gobioninae; however, the structure of the capsule differs in these two genera.

While in most members of the Gobioninae, one or two rows of teeth from the pharyngeal dentition, there is only one of the examined genera, *Hemibarbus*, which has three rows. This feature is more cyprinine than gobionine. The systematic position of *Hemibarbus* may have to be examined in greater detail. At present, I am not going to discuss the relative primitiveness of the single row or three rows of teeth on the pharyngeal bone. But I will only mention here that the teeth of *Gobiobotia* are differently shaped from those of *Saurogobio*. I have examined only a small specimen of *Gobiobotia* and it is known that the pharyngeal teeth change their shape with age.

It is thus obvious that the three genera *Saurogobio*, *Abbottina* and *Pseudo-*

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gobio resemble the catostomid *Catostomus* (and possibly Catostomidae) in possessing similar structures in the ethmoid region, the elongated median rostral, the ventral prevomerine flexure and process, a fontanel in the roofing bones and a degenerate or completely wanting supraorbital bone. In the weberian apparatus, however, the gudgeons differ from *Catostomus* and they (except *Saurogobio* and *Gobiobotia*) resemble more the Cyprininae.

If the Catostomidae are primitive forms[1], then the three gudgeons in showing a number of similar catostomid skeletal characters are probably also primitive and therefore closely related to the Catostomidae. Very likely, these three genera belong to a group of gudgeons still retaining a number of catostomid characters when the former superseded the suckers as envisaged by NICHOLS (1943). The other members of Gobioninae (except *Gobiobotia*) appear to show a number of cyprinine characters.

Gobiobotia is considered as exhibiting characters common to loaches and gudgeons by NICHOLS (1943). The distinguishing characters of the Cobitidae (RAMASWAMI 1953), viz., a) an united ethmoid-prevomer, b) an united orbitosphenoid, c) a pair of prepalatine ossifications, d) the dorsal ribs of the second vertebra taking part in the formation of the gasbladder capsule, e) uniformly one row of pharyngeal teeth, are not exhibited by *Gobiobotia* and hence, it cannot be a loach. In possessing a fontanel in the roof, an enlarged second preethmoid like *Chilogobio*, and a poorly developed subtemporal fossa, it resembles gudgeons like *Chilogobio* but not *Saurogobio*, *Abbottina* or *Pseudogobio*. A peculiar modification concerns the gasbladder; in appearance, it looks like that of a nemachiline loach but probably has arisen independently in this animal. While it is certainly not a loach, it may not merit the rank of being the type of a subfamily as has been done by Berg. It may however, be retained as a member of the Gobioninae with the understanding that we have *three* groups in this subfamily: one group embraces the genera *Saurogobio*, *Abbottina* and *Pseudogobio* showing affinities with the Catostomidae, the second group having *Gobiobotia* as its sole representative and apparently resembling the Nemachilinae (Cobitidae) in its gasbladder capsule and the third group, for the sake of convenience, will comprise all the other gobionine genera examined by me.

SUMMARY

1. The skull and weberian apparatus of twelve genera of Gobioninae (Cyprinidae) has been examined by me.
2. The three genera *Saurogobio*, *Abbottina* and *Pseudogobio* show elongated

[1] Eaton (1935) considers, from the view point of jaw mechanism, the catostomids to have descended from the cyprinids.

second preethmoid cartilages; in the other members studied, they are very much shorter. In *Catostomus*, the second preethmoid is elongated.

3. The median rostral is elongated in *Saurogobio*, *Abbottina*, *Pseudogobio* and *Gobiobotia*.
4. The anterior portion of the ethmoid-prevomer shows a ventral flexure in *Saurogobio*, *Abbottina* and *Pseudogobio* as in *Catostomus*. In *Gobiobotia*, it is not seen.
5. *Saurogobio*, *Abbottina*, *Pseudogobio* and *Gobiobotia* show fontanel or fontanelles in the roof as in *Catostomus*.
6. The supraorbital is either absent (*Saurogobio*, *Abbottina*, *Corieus*) or diminutive (*Gobio*, *Pseudogobio*); in other genera examined, it is well developed. In *Catostomus*, a supraorbital is absent.
7. In the occipital region, the occipital sensory canal passes through the supraoccipital in *Saurogobio*, *Abbottina*, *Pseudogobio* and *Gobiobotia*; in the other genera, it passes through the parietals.
8. One or two rows of pharyngeal teeth are more commonly met with in the Gobioninae; *Hemibarbus* is the only one with three rows of teeth.
9. The gasbladder is enclosed in a bony capsule only in *Saurogobio* and *Gobiobotia* but the structure of the capsule differs in them. The capsule of *Gobiobotia* apparently resembles that of the Nemachilinae (Cobitidae) but the second pair of dorsal ribs does not contribute towards the formation of capsular wall.
10. The weberian apparatus of the Gobioninae resembles that of Cyprininae; in *Saurogobio* and *Gobiobotia*, the transformator process of the tripus is absent on account of the development of a capsule.
11. *Saurogobio*, *Abbottina* and *Pseudogobio* in exhibiting a large number of catostomid features, probably represent a group of gudgeons more nearly related to the *Catostomidae*.
12. *Gobiobotia* is not a loach (Cobitidae) and shows certain specializations and it may be treated as a type of a group under Gobioninae; *Saurogobio*, *Abbottina* and *Pseudogobio* form a second group and the rest may conveniently be put under a third group.

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KEY TO ABBREVIATIONS

aee anterior extension of supraethmoid
af anterior fontanel
afl articular facet of quadrate for lower jaw
afp articular facet in entopterygoid for palatine
aft articular facet in the third centrum for the tripus
ale articular facet in the entopterygoid for the lateral ethmoid
anp anterior process of tripus
api ascending process of intercalarium
app anterior process of palatine
aps ascending process of scaphium
ari articular process of intercalarium
arp articular process of tripus
arp' articular process of preopercle
ars articular process of scaphium

bhy basihyal
bo basioccipital
bv basiventral

c cartilaginous projection
c1, c4 centra 1 and 4
c1', c2', c3' copulae 1, 2, 3
c23 united 2nd and 3rd centra
cb1, cb2 ceratobranchial 1 and 2
cb5 ceratobranchial 5 or inferior pharyngeal bone
chy ceratohyal
cl claustrum
co occipital condyle

dr1, dr2, dr4, dorsal ribs 1, 2 and 4

eb1—eb4 epibranchs 1 to 4
ef exooccipital fenestra
ehy epihyal
eo exoccipital
epa ethmoid articulation of palatine
epa' preethmoid articulation of palatine
epi epiotic
et ethmoid
ev4 posterior extension from fourth vertebral centrum

fea lateral ethmoid articulating surface with palatine
fep facet of palatine for articulation with entopterygoid
fr frontal

gb gasbladder

hb1—hb3 hypobranchs 1 to 3
hf hypoglossal foramen
hyh hypohyal
hyp hyomandibular facet for pterotic articulation
hys hyomandibular facet for sphenotic articulation
hy2 second hypohyal

if4 foramen for spinal nerve
int intercalarium
iol interorbital ligament

jf foramen for ix and x cranial nerves

lac lacrimal
le lateral ethmoid
lfa lateral facet on the prevomer
lo lateral opening in the gasbladder capsule

max maxilla

n nasal
na2—na4 second, third and fourth neural arches
ns23 united neural spines of 2nd and 3rd vertebrae
ns4 fourth neural spine

oc occipital sensory canal
oep outline of epibranchs
of optic foramen
ogc outline of gasbladder capsule
opa outline of palatine
ope outline of preethmoid
opi opisthotic
opt outline of posttemporal
opv outline of prevomer
os orbitosphenoid
os' os suspensorium
os4 outline of suborbital 4

pa par parietal
pal palatine
pas parasphenoid
pf posterior fontanel
pgb posterior gasbladder
php pharyngeal process
phy articular facet for hyomandibular in pterotic
pls pleurosphenoid
pmy posterior myodome
pmx premaxilla
ppm articular facet of maxilla with palatine
ppq posterior process of quadrate
ppv posterior process of prevomer
pre preethmoid
pro prootic
pr2 preethmoid 2
pr4 pleural rib 4
pt posttemporal
pte pterotic
pv prevomer

rpl ligament between rostral and rostral process of premaxilla
rpm rostral process of maxilla

rpp rostral process of premaxilla
r rostral

sc scaphium
sco sensory canal in opercular
se supraethmoid
shy facet for hyomandibular in sphenotic
so supraoccipital
so1—so5 suborbital ossicles 1 to 5
sor supraorbital
sp sphenotic
st supratemporal
stf subtemporal fossa

tc temporal sensory canal
te teeth
te3, te5 teeth rows of 3 and 5
tfo trigeminofacial openings
tp transformator process of tripus
tr, tri tripus

ixf glossopharyngeal foramen
xf vagus foramen

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AUTOTOMIZED TENTACLE CROWNS AS PROPAGATIVE BODIES IN PHORONIS

BY

LARS SILÉN

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The workers on Phoronis have long observed that these animals are very much disposed to throw off their tentacle crowns when the state of their surroundings becomes unfavourable. The phoronids possess strong regenerative powers. The various researches carried out in cases where the tentacle crown has been removed — spontaneously or by means of amputation — have almost exclusively concentrated on the regeneration which regularly turns the remaining body into a complete animal, whereas the fate of the tentacle crown has not been commented upon. The only exception that I have found in the literature is CORI's (1939, p. 124) brief remark that the amputated tentacle crown shows slight signs of regeneration at its severed end but that this effort comes to nothing. He concluded that thus "Die zwei Teilstücke der *Phoronis* nach der Autotomie oder der Amputation verhalten sich auf das Regenerationsergebnis verschieden".

During a stay in the summer of 1954 at Kristineberg Marine Zoological Station on the west coast of Sweden, the present author kept a number of mollusk shells containing pseudocolonies of *Phoronis ovalis* Str. Wright in comparatively small jars, the water of which was changed at irregular intervals. When an interval surpassed 24 hours, the tentacle crowns were thrown off in large numbers: apparently there exists a rather exact limit for the endurance of the species in unsuitable conditions and when this limit is reached, all the individuals contained in the same jar autotomize almost simultaneously.

In the tentacle crown just shed (fig. 1) the body consists of a quite small, abruptly severed stump. When undisturbed the tentacles are kept straight, their top ends curved slightly inwards. They are regularly arranged to form a narrow funnel. The entire body moves along, its severed end first, occasionally rotating round its longitudinal axis. The movement is slow and feeble. It does not suffice to keep the body floating in the upper water layers though it can occasionally raise it a little above the bottom, along which it normally glides. A quite slight blow with a pipette is sufficient to make it whirl up all over the jar. It can be assumed that the currents sweeping along the natural habitats of the species are sufficiently strong to keep such detached tentacle crowns floating in the same way as they presumably sweep away the young embryonic stages (cf. SILÉN 1954 b, p. 246). They show no clear reactions to light.

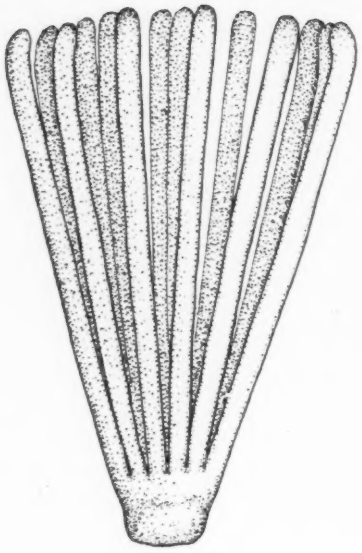

Fig. 1. *Ph. ovalis.* Just autotomized tentacle crown. 110 x.

Such autotomized tentacle crowns were collected and placed in finger bowls, 1—3 specimens in each; from these they were removed twice a day and put into bowls with a fresh supply of sea water (this is the same method as that used for the rearing of phoronid embryos, cf. SILÉN 1954 b, p. 225).

By this method the bodies were kept alive for up to three weeks. During the first eight days a development took place.

Already during the first day after the autotomy the originally flat amputation surface swells so that the body becomes hemispherical in shape (fig. 2). During the subsequent days the body grows still more. On the eighth day it has reached a length five or more times the original one (fig. 3). Of the body thus formed the anterior 2/5 retain approximately the original diameter, whereas the posterior portion is considerably thicker: the differentiation into the muscle portion and ampulla respectively of the complete adult is indicated. After the third day the tentacles become gradually shorter and thicker, finally reaching not half the original length but almost twice the original diameter.

Four days after the autotomy single tentacle crowns begin to adhere to the substratum, and two days later most of them have settled. If kept in a clean bowl, only a few of them settle but if pieces of mollusk shell are added to the bowl, about 75 per cent of them settle. They are then seen to prefer the pieces of shell to the glass bottom. They thus seem to show a tendency of the dependence on a special substratum for the settlement that is exhibited by the larva (SILÉN 1954 b). They take up an erect position from the very first and immediately start secreting a tube. If a specimen that has been attached for some days to an even shell surface, is removed, a small pit is seen at the

Fig. 2. *Ph. ovalis*. Horizontal section of tentacle crown 1 day after autotomy. Posterior end completely healed. 185 x. — *i* intestine; *rv* ring vessel.

place where its posterior end had been situated: the specimen had begun to burrow in the piece of lime.

Concerning the interior organization of the developing tentacle crown we have one previous item of information, i.e. CORI's (1939, p. 124) observation that the detached tentacle crown possesses "insofern eine Tendenz zur Regeneration, als sich die Enden der beiden Darmschenkel miteinander verbinden und ebenso auch die beiden Blutgefässe". (The name of the species examined was not stated.)

Indeed, the adult phoronid possesses an organization that forms, in relation to the place where the autotomy normally occurs, a quite unusually good fundament for a swift reorganization of the tentacle crown into a viable organism in spite of its small size. Though the autotomy takes place at such a level that only an inconspicuous portion of the "body" itself remains in connection with the tentacles, this small portion contains central or at least important parts of all the vital organs.

The anterior and posterior ends of the gut are contained in it. As stated by CORI, they very soon fuse into a coherent tube. In fact, this fusion seems to take place or, at least, to be already well prepared when the autotomy is performed.

The intact circular vessel is also situated there as well as, of course, the tentacle vessels issuing from it. It is easily seen that these elements are sufficient for a regular circulation, as the normal rhythm of the pulsation is re-established within the first hour after the autotomy. The erythrocytes are strikingly sparse immediately after the autotomy but then increase rapidly in number. Concerning the fusion stated by CORI to take place between the parts of the longitudinal vessels (three in number in *Ph. ovalis* according to LÖNÖY

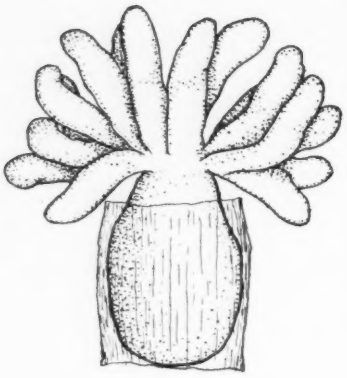

Fig. 3. *Ph. ovalis*. Settled tentacle crown. 8 days after autotomy, somewhat contracted. 110 x.

1953, p. 14) included in the tentacle crown portion, the present author has made no observations.

The nephridia remain in the tentacle crown portion and so does, of course, the respiratory organ represented, as it is, by the tentacles. So does the nervous ring as well, which contains the associative centres, etc. (SILÉN 1954 a). From the very first the autotomized tentacle crown exhibits the reactions normal to that body portion when still pertaining to the complete animal.

To the exterior differentiation of the regenerating metasoma into muscle portion and ampulla, there corresponds an interior differentiation (fig. 4). Eight days after the autotomy the body wall of the anterior metasoma portion is considerably thicker than that of the posterior part and shows traces of the longitudinal muscle strips beginning to form. In the epidermis of the posterior end numerous glands have appeared. They are of the type the secretion of which probably contains the limedissolving matter.

It may seem peculiar that the rate of differentiation gradually and distinctly decreases during the 6th—7th day after the autotomy, and the development finally comes to a complete standstill on the 8th—10th day. During the remaining days — up to 10 or more — during which the body was kept alive, it exhibits a slow shrinkage and, finally, signs of degeneration.

This phenomenon is, in all probability, artificial and can be ascribed to the laboratory conditions under which the research was carried out.

The tentacle crown portion contains no special storage tissues. Presumably only very little can be drawn from the other tissues for the benefit of the regeneration without endangering the welfare of the organism as a whole. (The strongly diminishing length of the tentacles which was remarked upon above, might possibly be interpreted as a shrinkage due to a removal of contained matters for use in other places. However, the tentacles get thicker simultaneously and it may be that their total volume does not diminish, in fact.) On the other hand, the swift reorganization of the alimentary canal,

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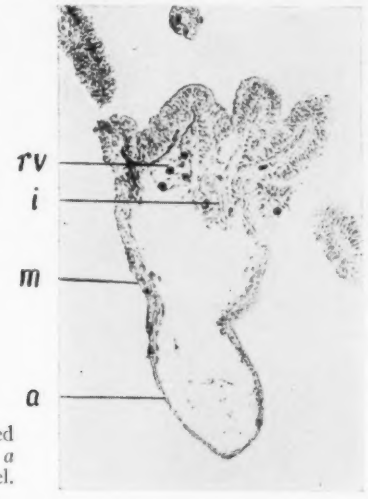

Fig. 4. *Ph. ovalis*. Oblique sagittal section of settled tentacle crown, 8 days after autotomy. 185 x. — *a* ampulla; *i* intestine; *m* muscle region; *rv* ring vessel.

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etc., indicates that the body is quite soon able to nourish itself. This possibility is supported by the observation that the tentacles really catch food particles, which are transported into the mouth. On thin sections contents are seen in the gut.

To use terms applied to pelagic larvae, the autotomized tentacle crown does not represent a lecitotrophic but a planktotrophic body. In the present case, it is thus appropriate to make a comparison with the results obtained in the rearing of phoronid larvae (SILÉN 1954 b). Lecitotrophic stages of the latter can be reared quite easily. The development of the planktotrophic stages, however, comes to a standstill about six days after they have been placed in laboratory conditions, probably owing to the lack of sufficient quantities of the suitable food. This food consists of peridinians, of which — to my knowledge — cultures cannot be made and a substitute for which has not been found in the case of the phoronids. Probably the same points of view can be applied to the case of the rearing of the autotomized tentacle crowns.

Nevertheless, the rearing of the tentacle crowns and their regeneration advanced so far in the experiments described that it seems to the present author that there can hardly be any doubt that their regeneration into complete animals would have taken place, had it not been for the deficiencies of the culture method employed.

The regeneration itself, as described above, shows every sign of being a normal phenomenon. The throwing off of the tentacle crowns under disagreeable conditions described by several authors, seems to have been observed exclusively in animals kept in aquaria; nevertheless, it is always taken for granted, without further discussion, that corresponding conditions with a

subsequent regeneration of the metasoma appear in nature as well. In the present connection it is of special interest that just in *Ph. ovalis* a throwing off of the tentacle crowns appears as a regular feature in the normal life cycle of the adult: it always takes place before the ova are shed (SILÉN 1954 b, p. 243). There are thus good reasons to believe that the phenomenon treated in this paper occurs in nature as well. The definite proof of its occurrence there will perhaps never be obtained, as it is difficult to imagine that it will ever be possible to observe in nature the coherent chain of the happenings here described.

An asexual propagation based on the strong power of regeneration, is well known to occur in the phoronids (cf. the survey given by SILÉN 1954 b, pp. 254—255) and is particularly pronounced in *Ph. ovalis* (HARMER 1917, MARCUS 1949). Previously it was only known to give rise to immobile new individuals resulting from the regeneration of autotomized metasomas and remaining in the immediate vicinity of their mother individuals. Now it seems as though it can give rise to free and mobile descendants as well, a formation of free propagative bodies that occurs (1) when in precarious circumstances the liberatable body portions escape leaving the non-liberatable portions to their doubtful fate, (2) regularly in connection with the sexual propagation.

It should be stressed that these statements are, as yet, valid for the single species *Ph. ovalis* only. The author did not succeed in obtaining a regeneration of autotomized tentacle crowns in *Ph. mülleri* or *Ph. pallida* (in *Ph. hippocrepia* he was not able to provoke an autotomy at all). It may be that the phenomenon described here is not of general occurrence in the phoronids and that this is the reason why it has escaped the attention of previous investigators.

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SUMMARY

Autotomized tentacle crowns of *Phoronis ovalis* Str. Wright first swim about freely, then settle and form a tube, preferably on a calcareous substratum, in which they start burrowing. As it proved possible to keep them alive for a rather long time, during which they reached a considerable degree of growth and reorganization, it was concluded that they represent free asexual propagative bodies normally occurring in the life of the species; these appear in connection with unsuitable living conditions as well as with the sexual propagation. No regeneration of autotomized tentacle crowns was obtained in other phoronids.

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STRUCTURE AND DEVELOPMENT OF REISSNER'S FIBRE IN THE CAUDAL END OF AMPHIOXUS AND SOME LOWER VERTEBRATES

BY

RAGNAR OLSSON

Zootomical Institute, Stockholm

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INTRODUCTION

Since the fine fibre called Reissner's fibre, running from the roof of the epithalamus through the brain ventricles and the central canal of the medulla in most vertebrates was first described by REISSNER in 1860, much work has been done as regards to its finer structure, the attachment to the subcommissural organ and its probable function. As regards to the termination of Reissner's fibre in the caudal end of the nervous system there are however rather few and often contradictory descriptions. The main aim of this work was to examine in more detail the structure and development of the fibre in this part of the body. The results of an investigation, now in progress, concerning the finer structure of Reissner's fibre and its relation to the infundibular and subcommissural organs, will be published separately.

MATERIAL

Branchiostoma lanceolatum. 14 tails of *Amphioxus* (about 45 mm in length) were orientated for cutting sagittal sections, but owing to the fact that the end

of the spinal cord is situated in the extreme tip of the tail, which is often more or less curved, all kinds of orientations between sagittal and transverse were obtained. The sections are 3, 4, 5, 6, 7 and 8 μ thick.

Of larval *Amphioxus* 22 series were cut in sagittal sections. The youngest specimens measured only about 0.5 mm, but, unfortunately, there were no stages available between these and the 3.0 mm larvae. All the other specimens were between 3.0 and 6.0 mm long, except for one that was 12 mm. The sections are 2-5 μ, most of them 3 μ thick.

Petromyzon fluviatilis. Sagittal and transverse sections (5 and 6 μ) from 8 old larvae and newly metamorphosed animals were used to show the conditions in the old stages.

Of the developmental stages, 36 specimens were cut, between 214 h old eggs and young ammocoetes 8 mm in length. The 214 h old eggs from Sweden seem to have reached about the same stage of development as those from Naples of 7 days investigated by v. KUPFFER (1894). All the egg stages are cut in 3 μ sections, while the young larvae in 4-6 μ.

Myxine glutinosa. Tails from four animals, about 160 mm in length, sagittally cut in 5, 6 and 7 μ thick sections.

Salmo salar. 6 young stages. The two youngest stages were in the late embryonic period, between 7.5 and 8 mm in length, with small pectoral rudiments. They were horizontally cut in 5 μ thick sections. One stage 8.5 mm, two stages 9.5 mm and one stage 12 mm, were all cut sagittally in 5 μ sections.

Clupea harengus. Tails from 4 young animals (about 50 mm). Sagittal and transverse sections, 7 and 10 μ.

Lebistes reticulatus. Sagittal sections of 4, 5.8—8.0 mm long animals, 4 and 5 μ thick.

Triturus cristatus. 2 tails of adult animals, 7 and 10 μ thick sagittal sections.

Xenopus laevis. 6 embryonic stages 1.5—2,5 mm, 8 larvae 3.0—9.0 mm and one larva 15 mm in length. Sagittal, 4—5 μ.

The tail from one old larva with the 8 mm long tail in resorption. Sagittal, 5 μ. The caudal region of a young frog without tail. Sagittal, 6 μ,

Pelobates fuscus. Tails from two old larvae, 32 and 45 mm in length. Sagittal, 4 μ.

Anguis fragilis. Tails from 5 newborn animals. One tail transversely cut in 5 μ sections, the others horizontally 6 and 7 μ.

METHODS

Several authors, especially NICHOLLS, have pointed out that Reissner's fibre often retracts during the preparation, due to the fact that the animal has been cut before the fixation has properly taken effect. In order to avoid this

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source of error all small specimens were totally embedded and sectioned so the fibre could be controlled throughout the body. The large specimens were fixed with uninjured nervous systems for a long time before the part to be investigated was cut off.

All the specimens were sectioned in paraffin and the young, yolk-laden stages were treated with amyl acetate as antemedium (BARRON 1934), which proved to be a useful method to avoid hardening otherwise caused by benzene and xylene.

The most useful stain for this study is the chrome alum haematoxylin — phloxine method according to GOMORI (1941). When staining reactions are mentioned in the following and the staining method is not described, the Gomori method has always been used. As a control method the paraldehyde fuchsin — pikroindigo carmine — trioxyhematein stain, according to GABE (1953), was applied and in some cases the periodic acid-Schiff reaction, according to HOTCHKISS (1948, in EVERSON PEARSE 1954). The Schiff solution was always prepared with thionyl chloride, according to BARGER and DE-LAMATER (in EVERSON PEARSE), which proved to be a rapid method that gave good results. The same Schiff reagent was also employed for a Feulgen reaction.

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OBSERVATIONS

ADULT *AMPHIOXUS*

For a long time it has been known (QUATREFAGES 1845) that the spinal cord of *Amphioxus* terminates in the caudal end as a kind of ampulla, which is formed by a single cell-layer and varies greatly in size, form and position (RETZIUS 1891). NICHOLLS (1912 b) believes that this terminal sinus has "an apparent terminal neural pore, directed, as in young ammocoetes, dorsally."

Some earlier authors (WOLFF 1907, KRAUSE 1923) have incidentally mentioned a Reissner's fibre in the central canal of *Amphioxus*. OLSSON and WINGSTRAND (1954) newly investigated the fibre and found that the anterior end is connected with the infundibular organ in the floor of the brain vesicle. In the tail it terminates as an irregular widening in the terminal ventricle.

The terminal portion of the spinal cord is a rather long *filum terminale* with only ependymal walls. In the caudal end it forms, in most of the specimens, the above-mentioned ampulla, the walls of which entirely surround the terminal ventricle. No trace of a neural pore, as suggested by NICHOLLS, can be seen. Not two of the examined specimens have these structures alike. They range from only a slight dorsal bending of the terminal portion of the spinal cord to very conspicuous ampullae. In two cases they are pronouncedly asymmetrical. In one of them there is a small second ventrally directed ampulla lateral

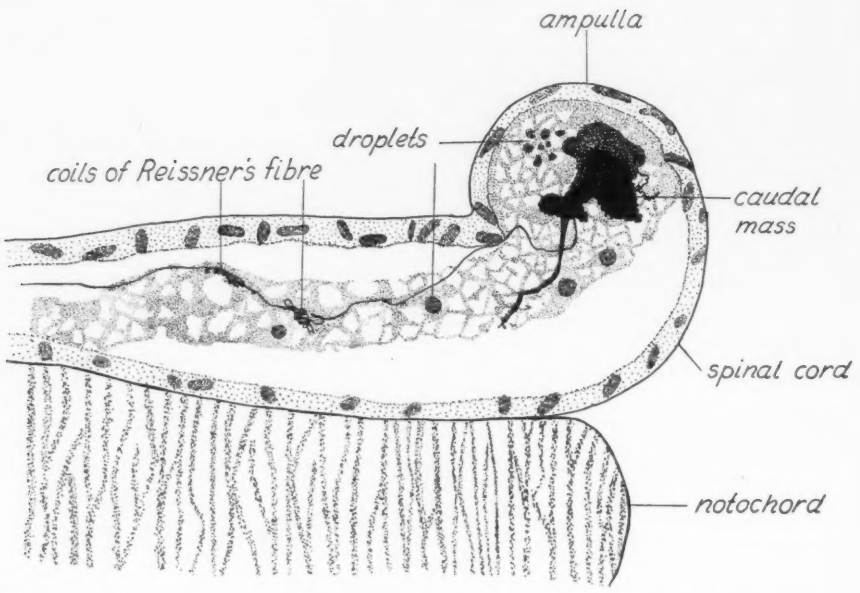

Fig. 1. *Amphioxus*. Semi-diagrammatic median reconstruction of the caudal portion of the spinal cord in an adult animal. 700 x.

to the tip of the notochord, in the other case the ampulla is not situated straight above the notochord but is displaced somewhat laterad.

Reissner's fibre can clearly be seen in the *filum terminale,* where it has a dorsal position caudally. In the periodic acid-Schiff sections it is difficult to decide whether the fibre reacts positive or not — because of its thinness. It is, however, even in these preparations, easy to follow its course in parts because of small adhering granules, probably remains of the coagulated ventricle fluid. As described by OLSSON and WINGSTRAND, fine threads can often be seen between the ependymal cells and the fibre. It is very difficult to decide whether they are ependymal cilia, but, at least in some cases, this seems improbable as they give the impression of anastomosing and branching. In periodic acid-Schiff stained sections small, but intensely red, droplets can often be seen adhering to these threads.

In a few specimens, the fibre makes some small coils far in front of the ampulla (fig. 1), but usually it has a straight course. It is interesting to note that in all specimens the fibre keeps very close to the ependymal fold that borders the ampulla in the dorsal wall.

In the ampulla, or in the caudalmost part of the spinal cord, where no ampulla can be distinguished, the fibre may lie close to the walls or quite free in the ventricle. In some cases it coils again and then gradually becomes thicker. This thickened part usually stains lighter, and thus seems transparent. In the

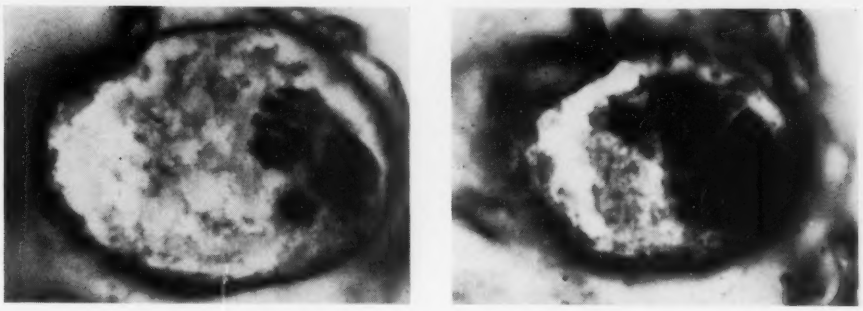

Fig. 2. *Amphioxus*. Two adjacent transversal sections through the ampulla. In the left section (the anterior) mainly fibre coils in the posterior an irregular mass. Gomori's haematoxylin, 7 μ, 950 x.

dorsal part of the ampulla the fibre suddenly changes to a large and heavily dark-stained mass, which apparently is identical with the structure which NICHOLLS has called the "terminal plug" in other animals.

This name is not very relevant, however, and "terminal mass" would better indicate the form and nature of this structure. It seems better to avoid the usage of this name as it may be confused with the *massa terminalis* (STERZI 1907), i.e. the mass of juvenile cells that in young specimens lies caudally to the spinal cord and notochord. In this paper the terminal part of Reissner's fibre, when forming such a structure, will be referred to as the *caudal mass* (of Reissner's fibre).

This caudal mass is seldom formed alike in different specimens. Sometimes it seems to consist mainly of the entangled fibre, but among the coils an extremely fine-granulated material that shows the same staining properties as the fibre can often be seen. In other cases there are but few fibre coils and the entire caudal mass consists of this secretion-like aggregation (fig. 2). The caudal mass lies, usually, close to the dorsal wall of the ampulla, and in one case it even seems to enter the wall and spread among the ependymal cells. The subcutis, outside the ampulla, is blue in colour in the chrome alum haematoxylin preparations. It is impossible to decide whether the caudal mass also penetrates the walls of the ampulla. A few blood — vessels are situated in the subcutis, but they cannot be connected with a possible outflow of substance from the ampulla. The periodic acid-Schiff preparations give positive reaction for the caudal mass and part of the inner border of the dorsal wall of the ampulla.

In the specimen reconstructed in fig. 1 a short second fibre can be seen between the caudal mass and the wall of the ampulla. The ventral end of this tightly coiled fibre terminates and divides into two minute branches between the ependymal cells, while its dorsal end gradually thickens and is converted into the caudal mass.

The ventricular content is greyishly stained except near the caudal mass where it often takes a slight red tone from the phloxine. In this fine-granulated coagulum clear red globules are often seen, varying in size from minute droplets up to the size of the ependymal nuclei. Except for the larger of these globules, nothing in the terminal ventricle can be interpreted as free cells. In some cases the smaller types of red droplets are especially crowded around the caudal mass.

LARVAL *AMPHIOXUS*

According to BOEKE (1908) the infundibular organ is already present in very young larvae (1.5 mm). If Reissner's fibre is produced by the infundibular organ as supposed by OLSSON and WINGSTRAND (1954) it may be possible that even the fibre is an extremely early structure during the development. The 0.5 mm stages proved to be too young to show these structures. In all the other specimens stained with chrome alum haematoxylin and paraldehyde-fuchsin the infundibular cells, though only a few in number, are easy to find as they contain intensely stainable granules. There is also a Reissner's fibre in all of them that is seen in a few cases to be in contact with the infundibular organ by means of a brush of tiny fibres. As a result of the minute thickness of Reissner's fibre it is often difficult to observe it, particularly in thick sections (fig. 3 and 4). The fibre has the same dimensions as the other fibrillar constituents of the central canal and the terminal ventricle. It may be very difficult to recognize it in oblique sagittal sections that only show a short portion of the fibre.

In the 3 mm stage the spinal cord bends round the caudal end of the notochord. In this stage there is no longer any communication between the neural and the gut tubes through a neurenteric canal, but only a few cells separate the two from each other (fig. 3 and 4). In the entire terminal portion the central canal is slightly widened and may thus be called a terminal ventricle. It is possible to follow Reissner's fibre as far as to the end of the spinal cord near the anus. There it suddenly thickens and terminates as a caudal mass consisting of blue stained drops. A single clear red droplet of the same appearance as those described in the adult animals can be seen near the caudal mass. In other specimens neither the terminal ventricle nor the caudal mass is very conspicuous. In most cases, however, blue stained material adheres to the walls of this part of the central canal. As it gives the same staining reactions and is situated in the same position as the caudal mass, but nowhere else in the spinal cord, it seems probable to consider it as a form of a caudal mass, i.e. the termination of Reissner's fibre.

In a 4.3 mm stage the spinal cord does not ventrally reach so far around the end of the notochord. It terminates as a distinct terminal ventricle in the

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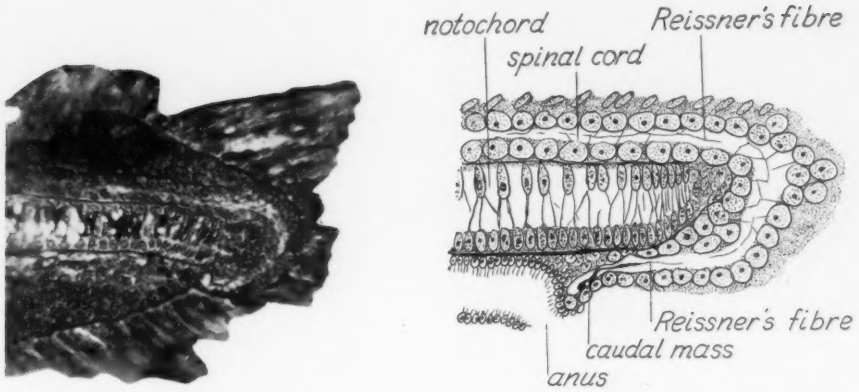

Fig. 3 and 4. *Amphioxus*. Median section and semi-diagrammatic reconstruction of the tails of 3 mm larvae. Photo: Gomori's haematoxylin, 4 μ, 500 x.

centre of which the end of the fibre forms a caudal mass. This mass is connected by means of threads with droplets and a blue stained layer coating the walls of the ventricle. A few of the clear red droplets and some of the pale red fine granulated coagulum can also be seen.

In the 4.5 mm stages the spinal cord is still shorter, and the terminal ventricle is situated near the caudal end of the notochord (fig. 5). One of these specimens was used for a periodic acid-Schiff reaction. As in the adult animals this method does not clearly stain Reissner's fibre in the central canal and terminal ventricle, probably because of the minute dimensions of the fibre. The walls of the terminal ventricle are, however, stained intensely red and the caudal mass gives the same reaction. Even the cells in the vicinity of the terminal ventricle are surrounded by a substance which gives a strong positive reaction.

All these larvae of about 4 mm length are essentially alike. One specimen, however, is a curious exception. Reissner's fibre here terminates as a swelling of some length, already in the central canal far in front of the tail. The fibre is distinctly visible only in front of this displaced caudal mass, but not behind it. In this specimen the caudal end of the spinal cord has only a slight curve ventrad. It reaches caudally about as far as the notochord and there it ends as a small terminal ventricle. This is quite devoid of contents in the preparations. A few minute, but intensely blue stained, granules are situated in its walls.

In a 5 mm stage the *filum terminale* does not reach behind the notochord. In its caudal end the first signs of an ampulla now appear. There is no distinct caudal mass, but the walls and the cavity of the ampulla contain many granules of the same appearance as those described in the previous stage. The granules stain in the same manner as those of the infundibular cells, but they are somewhat larger. Another specimen of the same size and the 5.5 and 6.0 mm

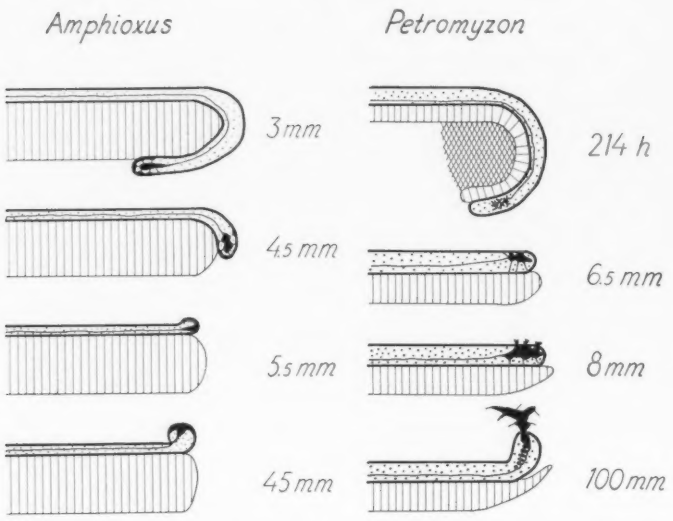

Fig. 5. Diagrammatic reconstructions of developmental stages of *Amphioxus* and *Petromyzon*. *Vertical lines:* notochord. *Crossed lines:* yolk. *Dotted area:* neural tube. *Black:* Reissner's fibre and caudal mass. Not drawn to scale.

stages have a pronounced caudal mass as well as granules (fig. 5). Here, the caudal mass often forms a thin layer covering part of the internal ampulla walls.

OLD LARVAE AND NEWLY METAMORPHOSED ANIMALS OF *PETROMYZON*

STUDNIČKA (1895) described the caudal end of the spinal cord in *Petromyzon* as a thin-walled terminal sinus, communicating with the surrounding tissues by means of a neural pore. Later (1899), he studied the structure of Reissner's fibre in this part. It is usually coiled in *ventriculus terminalis,* but caudally the fibre-form gradually disappears. The mass originating from it can even be traced in the surrounding connective tissue "wie ausgegossen". KOLMER (1921) gives a similar description. NICHOLLS (1912 b) considers all cases with an entangled fibre as artifacts. The fibre is normally stretched in the cavities of the central nervous system and posteriorly attached to the fibrous tissue of the meningeal sheaths.

The specimens examined do not show a well-defined terminal ventricle, but the cavity of the caudalmost part of the spinal cord is widened. The end of the spinal cord is often directed dorsally (fig. 6) and is then suggestive of the ampulla of *Amphioxus.* However, an important difference is the terminal pore in *Petromyzon,* through which the terminal ventricle communicates with the surrounding tissues.

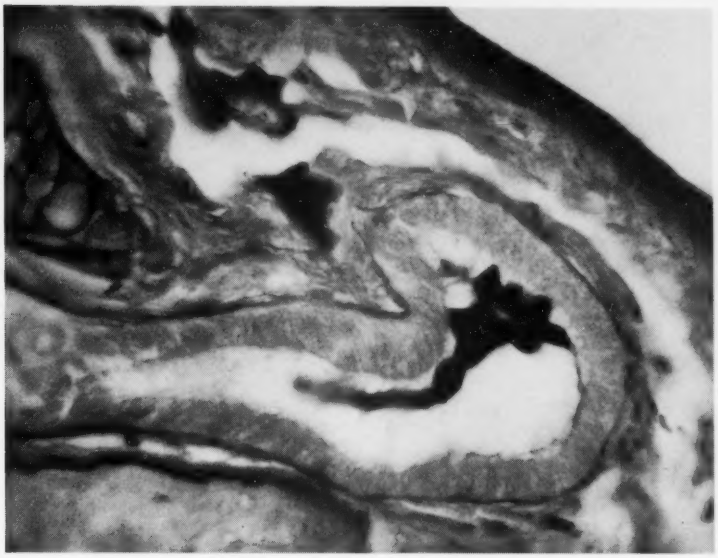

Fig. 6. *Petromyzon.* Sagittal section through the extreme end of the spinal cord with the caudal mass. Note the mass-substance in the connective tissue dorsally to the spinal cord. Gomori's haematoxylin, 6 μ, 530 x.

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As in *Amphioxus* the caudal portion of the fibre often coils. In the extreme end of the spinal cord the fibre looses its filamentous nature. It broadens and often stains lighter, and gives the impression of being fluid. This is the caudal mass (fig. 6 and 7) that seemingly flows through the neural pore.

It is very difficult to establish in what degree the terminal mass is enclosed by the meninges, i.e. is situated in the terminal ventricle. At least in some cases the caudal mass with certainty reaches far into the surrounding connective tissue (fig. 7). Here it often gives off short branches intermingling with other elements in the connective tissue that give the same staining reactions, mainly elastic fibres, thus making it impossible to follow their destination. The caudal mass gives a strong impression of being a fluid that runs freely in the connective tissue. It can often be seen to flow around cells, thus giving the impression that the caudal mass in itself has a cellular structure. These enclosed cells are usually the normal constituents of the connective tissue or endothelial cells of the blood capillaries, but even some macrophages may be found among these cells. There are many small blood vessels in this region, but nothing indicates the entering of the mass substance into vessels.

One series shows plainly the ciliated ependymal cells lining the central canal. Each cell carries several cilia that are, together with the basal corpuscles, clearly red stained by the phloxine. The cilia are rather short and do not by far reach Reissner's fibre, when it is situated in the centre of the terminal ventricle.

Fig. 7. *Petromyzon*. As fig. 6 but transversely cut through the caudal opening of the spinal cord (*). A rest of the fibre can still be seen in the central canal, the remaining fibre-substance leaving the nervous system (arrow). Gomori's haematoxylin, 5 μ, 640 x.

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Besides the ependymal cilia, fine threads here and there join the fibre and the ependymal wall. They are apparently identical with the similar structures of *Amphioxus* and differ considerably from ependymal cilia. In a few cases Reissner's fibre forms small bulges at the points where these threads come in contact with the fibre. Even at the highest magnifications the two structures seem to be confluent. In the periodic acid-Schiff sections Reissner's fibre and the caudal mass show a clearly positive reaction. The above-mentioned fine threads are too tiny to be stained, but many positive-reacting small droplets adhere to them (fig. 8). These droplets also seem to stick to the ependymal cilia but are never found free in the central canal. They do not vary greatly in size and can be found rostrally all through the central canal as far as the examination reaches. In this specimen the caudal mass is already formed in the dorsal curve of the spinal cord, and no droplets are found in the region of the caudal mass.

LARVAL *PETROMYZON*

KOLMER (1921) found that Reissner's fibre in a 35 mm ammocoetes caudally terminates with a slight swelling. AGDUHR (1922) states that there is apparently a fibre even in the smallest larvae, but he had no available material smaller than 25 mm in length. In the same year SCHAFFER (1922) pictures a median

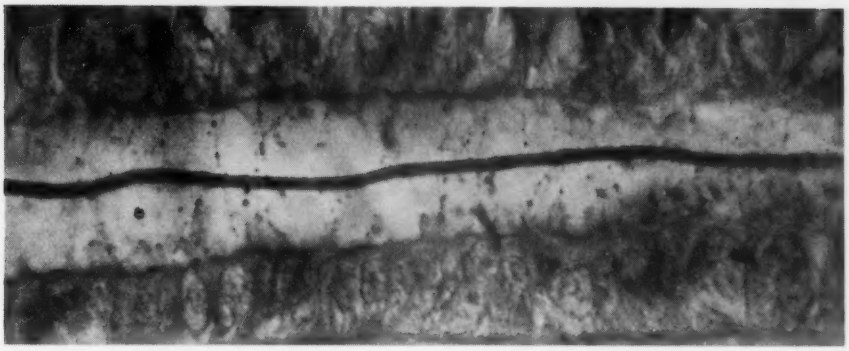

Fig. 8. *Petromyzon.* Reissner's fibre (arrow) and some small droplets are the only periodic acid-Schiff positive matter in the central canal. Sagittal, 6 μ, 680 x.

section of a 7 mm larva in his textbook of histology. In this illustration he has drawn a Reissner's fibre in the central canal. The fibre ends among the dorsal wall cells near the termination of the spinal cord.

The youngest studied specimens were eggs labelled 214 h. Already at this stage there is a Reissner's fibre though only recognizable in the hind part of the neural tube. There it is stained distinctly blue by the chrome alum haematoxylin. In young stages the fibre has not the usual even surface, but is rather rough (fig. 9). This roughness increases caudally to a point where the fibre rather has the appearance of being changed to a liquid running around the cells lining the central canal and forming a delicate network with the same staining properties as the fibre (fig. 5). There is no trace of this substance further back, and it seems reasonable to consider the network as a juvenile caudal mass.

In the slightly older stages this meshwork is never missing. The fibre usually can be seen distinctly in the central canal and is often perfectly smooth and threadlike. In a 246 h specimen the central canal is somewhat widened caudally and forms a small terminal ventricle in which the juvenile caudal mass is situated.

In a 4.5 mm larva some cells in the brain roof, just behind the parietal pouch, are well recognizable as subcommissural cells. They contain blue stained droplets and in the ventricle near the surface of these cells are some fine blue threads, apparently a part of the Reissner's fibre apparatus. Signs of a subcommissural organ are indicated already in younger stages as will be described in detail in a work now in progress dealing whit the development of the subcommissural organ and the fibre. At this stage the caudal termination of the fibre is still of the network type.

In the 6.5 mm and older specimens there is a more concentrated caudal mass, suggestive of that of the adult animal. The caudal portion of the central canal is

more widened, and a long *ventriculus terminalis* can be distinguished. It is interesting to note that the dorsal wall cells always are arranged rather irregularly in the region above the mass. At the 6.5 mm stage, part of the mass lies close to the dorsal wall and is also seen between these cells but without penetrating the wall (fig. 5). Even in the older larvae the caudal mass always shows this affinity to the dorsal wall. From the 7 mm stage on, the mass substance penetrates the wall between the mentioned irregularly arranged cells (fig. 5, 10). The mesenchyme outside this place shows generally some dark stained material, apparently parts of the caudal mass.

The fine blue threads described in *Amphioxus* and old *Petromyzon* stages are usually present even in these stages. In a 9 mm larva the difference between the threads and the ependymal cilia is clearly visible.

MYXINE

SANDERS (1894) and STUDNIČKA (1895, 1899) described the termination of Reissner's fibre in *Myxine* as a strange colloidal mass in the "*terminal sinus.*" This mass is also found outside the spinal cord in the surrounding connective tissue. NICHOLLS (1912 b) investigated two animals and in both of them he found the fibre ending as an enormous mass formed by the coiled fibre. He considers that this concentration of fibre in the caudal end is an artifact, caused by the fibre being retracted from a long distance in the central canal. Accordingly he states that his, as well as SANDERS' and STUDNIČKA's material might have been cut or else injured before fixation had taken effect. KOLMER (1921) describes a case with a tangled mass but pictures one with an amorphous caudal mass in his diagram. The mass is situated completely within the terminal ventricle and also KOLMER believes that a coiled condition of the fibre is an artifact.

In the specimens used for this investigation, the thick fibre runs in a straight course throughout the central canal and into the terminal ventricle where it is transformed into a large, amorphous caudal mass. The minute threads in the central canal that have been mentioned in *Amphioxus* and *Petromyzon* are frequently to be seen also in *Myxine*. As their position in the central canal often is perpendicular to Reissner's fibre, they will be referred to in this work as "the perpendicular threads." As in *Petromyzon,* periodic acid-Schiff positive drops adhere to the threads in the central canal but not in the terminal ventricle, where the only positive-reacting material is the caudal mass.

The caudal mass is very large and gives the impression of partly having run out of the terminal ventricle into the interspaces of the meninges and the surrounding loose connective tissue (fig. 11, 12).

Free cells are often found in the terminal ventricle. Sometimes they are entirely surrounded by the caudal mass. The nuclei of these free cells are

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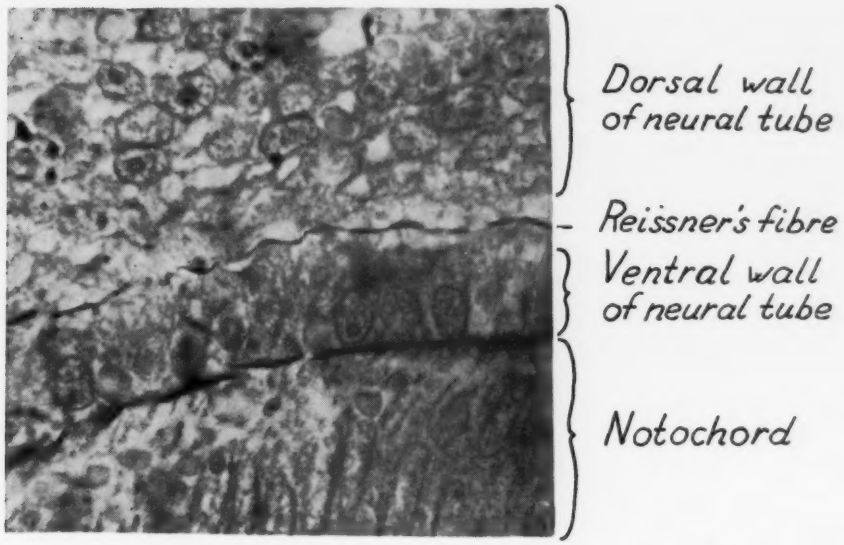

Fig. 9. *Petromyzon*, larva 246 h. old. Sagittal section through the caudal part of the neural tube, with a minute Reissner's fibre in the central canal. Gomori's haematoxylin, 3 μ, 1.140 x.

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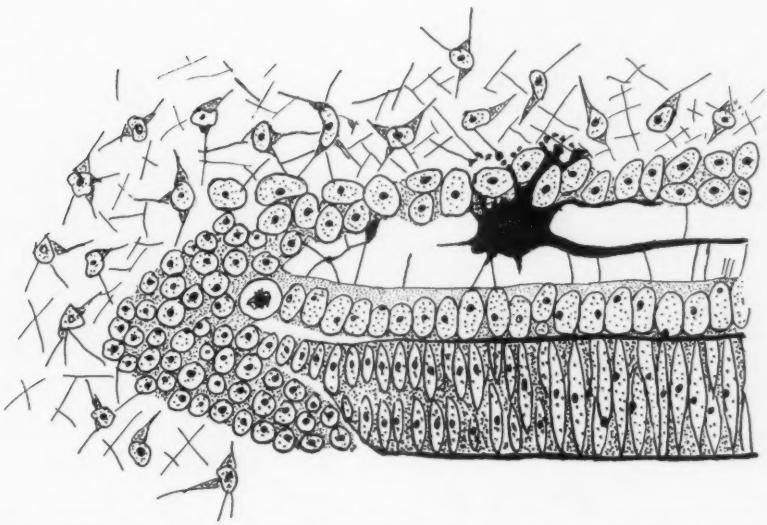

Fig. 10. *Petromyzon*, about 7,5 mm. Semi-diagrammatic reconstruction of the caudal end of spinal cord and notochord. Caudal mass penetrates the dorsal wall. 800 x.

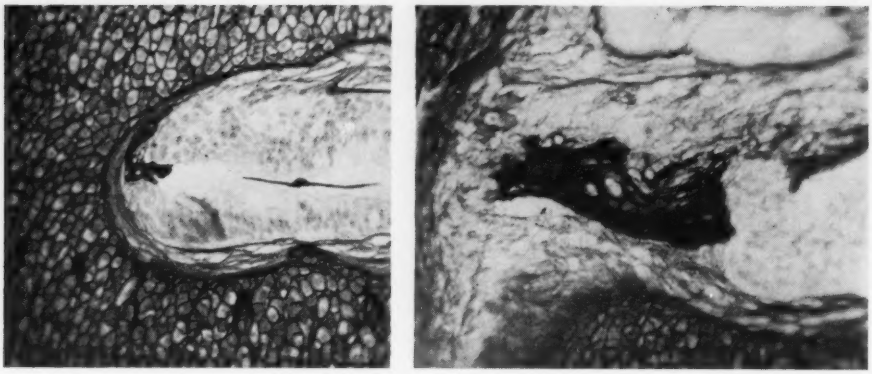

Fig. 11 and 12. *Myxine.* Median section (left) through the extreme end of the spinal cord, with part of the fibre and the caudal mass enclosed by the meninges and the cartilage plate. Right, a slightly parasagittal section of the same region. The caudal mass has free passage into the connective tissue. Gomori's haematoxylin, 7 μ, 138 x.

similar to those of the ependymal cells, and it is possible that some of the ependymal cells can be liberated in the ventricle.

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TELEOSTS

In *Gasterosteus* NICHOLLS (1912 a) followed Reissner's fibre through the long *filum terminale* into a terminale sinus "in which, in some instances, it expands into a conical terminal plug" — "and becomes inserted into that part of the wall of the sinus terminalis which is constituted by the meningeal sheath." KOLMER (1921) states that the fibre of *Gasterosteus* ends with a small swelling within *filum terminale.* He found the same condition in *Salmo,* although the terminal part of the fibre here reaches out of the spinal cord as a result of degeneration of the caudalmost cells of the spinal cord.

All the examined specimens of *Clupea* and *Lebistes* are very much alike as to the termination of Reissner's fibre. The long and slender *filum terminale* is bent dorsad behind the last neural spine (fig. 21). The central canal in this part is narrow and the caudal tip only slightly widened, so there seems to be no reason to speak of a *ventriculus terminalis.* Reissner's fibre has a straight course all through the central canal. In one *Lebistes*-series a few free cells can be seen in the central canal, some of them being attached to the fibre. This condition, however, seems only to be accidental as there is no relation between cells and fibre. As the cells are so scarce and lacking in most series it seems improbable to consider them as parts of the fibre.

The terminal foramen does not seem to be a definite structure. It rather gives the impression of being a rupture of the spinal cord wall somewhere near

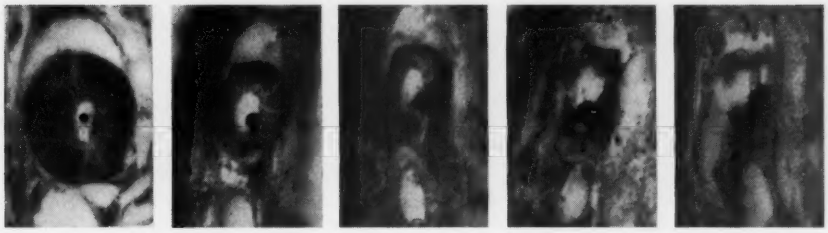

Fig. 13. *Clupea.* A series of transversal sections through the end of filum terminale. To the right 4 adjacent sections through the extreme end. Note the black fibre in the central canal, gradually transforming into a mass and leaving between the desintegrating wall cells. Gomori's haematoxylin, 10 μ, 750 x.

the caudal end. Sometimes the fibre begins to form a caudal mass already in this portion. Part of the mass then runs through the scattered ependymal cells into the surrounding connective tissue (fig. 13). In other cases the fibre penetrates the walls while still thread-like and forms a caudal mass or tangle in the connective tissue. The tangled part of the fibre is often thicker than the straight part in the central canal and the last coils often have the appearance of being a fluid rather than a solid body. There seems to be all intermediate stages between the fibre and the amorphous, fluid-like caudal mass. It is interesting to note that in cases with a large tangle there is seldom a pronounced caudal mass and contrarywise.

It is impossible to trace the fate of the caudal mass behind the spinal cord. It is discharged in the loose connective tissue between the last hypural and the last dorsal spine. Sometimes there are a few macrophages in this region, but there is no distinct gathering near the mass. In one specimen, part of the caudal mass runs along a capillary for some distance but this seems to be a coincidence.

A few young stages of *Salmo* were examined. In the two youngest specimens (7.5—8 mm), Reissner's fibre is minute but very distinct and intensely stained. In one specimen the caudal part of the spinal cord has a slight widening and suggests a terminal ventricle. The fibre terminates here as a longish caudal mass. Between the mass and the ependymal walls, many fine threads can be seen. In the region dorsal to the caudal mass the wall cells are scattered and give free passage for the mass substance to enter the surrounding mesenchymal tissue. The substance can be traced for some distance in the mesenchyme in shape of small drops showing the same staining reactions as the mass and decreasing in number with the distance from the point of exudation. In one of these two young specimens there can be seen, together with the caudal mass in the central canal, some cellular material that may be the remains of dorsal wall cells. Any living free cells cannot be seen here. Both specimens have subcommissural organs producing stainable secretion, but the fibre cannot be observed in the brain cavities.

The older embryos resemble essentially the two youngest stages in this respect. The scattering of the caudalmost dorsal wall cells is sometimes very pronounced as for instance in the 8.5 mm stage, where the hindmost part of the spinal cord consists of only floor and side walls. The caudal mass is often a very large structure, part of which runs out of the spinal cord dorsally in the same manner as by young ammocoetes (fig. 10). From the 9.5 mm stage on, Reissner's fibre can clearly be seen even in the brain cavities, and therefore be observed all the way from the subcommissural organ to the caudal mass.

TRITURUS

According to EBERL-ROTHE (1951) Reissner's fibre in *Triturus* terminates as two branches, one of which ends in *filum terminale*, while the other enters the connective tissue outside the spinal cord.

Filum terminale is rather long and its cells are apparently unciliated. As in teleosts there is no clear indication of a *ventriculus terminalis*. Reissner's fibre forms a caudal mass, part of which leaves the spinal cord between some displaced dorsal wall cells, in about the same manner as described above in *Salmo*. In the connective tissue the caudal mass can be traced only for a short distance in the vicinity of the place of exit. The rest of the caudal mass in the hind portion of the filum forms a very liquid-like structure with the appearance of a blue foam. This dorsal outflow of mass substance and the remains in *filum terminale*, probably correspond to the two branches of the fibre described by EBERL-ROTHE.

XENOPUS AND *PELOBATES* TADPOLES

According to EBERL-ROTHE (1951) the caudal part of Reissner's fibre in *Pelobates* is "kolbig vedickt" and connected with the ependymal cells by means of fine fibrillae. She considers the fibre to be cellular and pictures nuclei in it. KOLMER (1921) investigated two species of *Rana* and remarked that the fibre must be a very early structure. Even in 7 mm long larvae he found an extremely tiny fibre. He pointed out that the fibre terminates freely without being in contact with the ependymal walls or leaving *filum terminale*.

The youngest stages used for this investigation were two embryos of *Xenopus*, 1.5 mm in length. It was not possible to trace a Reissner's fibre in these specimens, but the neural tube seems to be in an intense secretory activity. Here as well as in the gut and in other places in the embryo, a number of cells can be found producing granules that become stained dark blue by the alum haematoxylin.

Of three 2 mm examined embryos two had a minute but clearly identifiable fibre. In the third specimen this structure could not be discerned, but the

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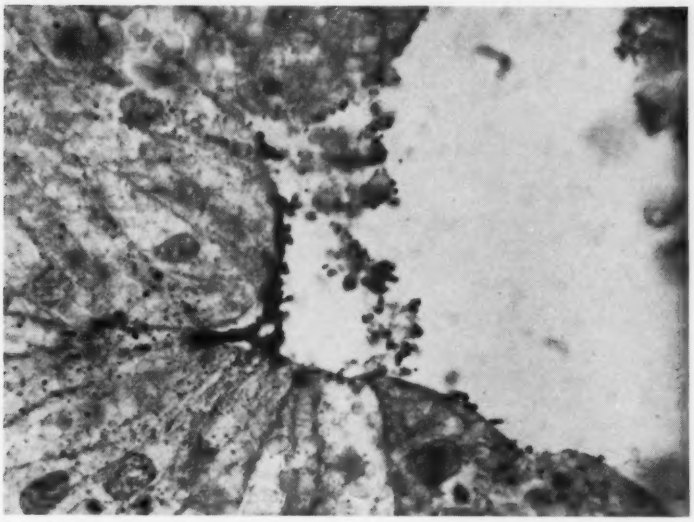

Fig. 14. *Xenopus*, 2 mm. A plug of alum haematoxylin-positive material where the neurenteric canal opens into the gut. The cavity is dorsad continued into the fore-gut and ventrad into the blastopore. Note the abundance of granules in the cells. Gomori's haematoxylin, 5 μ, 600 x.

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neural tube contained an amount of blue material, possibly a product of the granule-secreting cells. It is interesting to note that all these specimens have a plug of the same blue material, at the place where the neurenteric canal enters the hind gut (fig. 14).

No doubt the fine thread found in the central canal of two of the embryos is a Reissner's fibre, as is shown in a series of older stages where there is a well developed fibre always in the same position and with the same staining properties. It only increases in diameter in older specimens, and the 2 mm embryos represent the youngest stage where it can be seen. About half way of the neural tube, the fibre is but faintly stained and further in front of this place it cannot be traced. However, in the hind part, it stains much better, apparently without being thicker, and is here rather easy to follow. In one of the specimens the fibre cannot be discovered in the hindmost part of the neural tube. There is no trace of a caudal mass. In the other specimen Reissner's fibre is seen entering the neurenteric canal where it can be followed to a place near the opening into the hind gut. Here it suddenly disappears, without any mass-like swelling.

From the 3.0 mm stages on, the fibre can be traced all through its course in the central canal. As at the previous stages it enters the neurenteric canal and passes into the hind gut. In the gut it seems to be slightly thicker than in the

neural tube, but is rather difficult to follow because of yolk-cells having been pushed into the gut by the microtome knife. In the gut it is directed craniad but is suddenly lost among the yolk-cells.

At this stage an *organon subcommissuralis* region can be recognized. The cells are already typical with their basally situated nuclei, but they do not contain alum haematoxylin positive granules. In the ventricle, near this cell-layer, is, however, a small tangle of positive threads, apparently a product of secretion. In the ventricle, not far from this place, the first trace of Reissner's fibre can be seen. From here it can be followed through the brain ventricles and back.

At the 3.5, 4 and 5 mm stages there are still no visible granules in the cytoplasm of the subcommissural cells. Reissner's fibre is now distinct even in the brain ventricles. Close to the subcommissural organ many short and tiny threads can be seen irregularly arranged.

In the 3.5 mm stage, the fibre enters the neurenteric canal and may even be found for a short distance in the gut diverticulum. In the 4.0 mm specimen the fibre bends in a ventral direction around the extreme end of the notochord. Here, behind and slightly ventral to the notochord, it gradually looses its character of a smooth fibre and becomes rough and "spiny." This portion looks very much like the juvenile caudal mass in embryos of some of the other examined animals. As this part of the fibre apparently is the end, it seems reasonable to consider it as a lengthened caudal mass. Even at the 5 mm stage the neurenteric canal is seemingly hollow. The terminal part of Reissner's fibre is only faintly stained in this preparation, and therefore impossible to trace to the end.

A 6 mm specimen represents the youngest stage where alum haematoxylin positive granules can be seen in the subcommissural cells. The granules are situated basally to the nucleus, as has been described by many authors. Even in this stage there is no distinct caudal mass but the termination of the fibre has a striking resemblance to that of some of the young *Petromyzon* and *Salmo* specimens. The fibre gradually looses its smooth thread-like shape, the number of perpendicular threads increases and an elongated mass almost fills the caudal part of the cavity. As in the two above-mentinoned fishes, some of the dorsal wall cells in the caudal part of the neural tube are slightly disarranged. Between these cells caudal mass substance can be seen passing from the neural tube into the surrounding tissues.

In a 15 mm long larva the very long and thin-walled *filum terminale* terminates in the extreme end of the tail as a kind of ampulla that suggests the condition in *Amphioxus* and *Petromyzon*. This widened portion of the filum apparently lacks ependymal cells in the hind part of the wall. The other wall cells of this ampulla are few and irregularly arranged and therefore give free

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passage for the caudal mass. Evidently very much of the mass substance has passed this way and in the preparation stained the surroundings deep blue. The caudal mass is rather large.

The cytoplasm of a few of the wall cells contains alum haematoxyline positive granules. In every other respect they resemble very much the ordinary ependymal cells. Some blue droplets can also be seen in the terminal ventricle together with remains of cells. All larval specimens of *Xenopus* with a growing tail seem to resemble this stage very much.

In addition to the *Xenopus*-specimens two tails of *Pelobates* were examined. The purpose was to find the nuclei in Reissner's fibre described by EBERL-ROTHE (1951) in sections of this animal. According to her measurements the diameters of these elongated nuclei are 10.4 μ x 5.5 μ. Though a very careful examination was made as far as the fibre reached in the sections, it was not possible to find any nuclei in the fibre of any of these specimens.

In one of the specimens the caudal end of the *filum terminale* is slightly widened and opens dorsally through a pore. A small caudal mass is found in the terminal ventricle but most of the fibre substance passes through the pore in a manner that has a striking resemblance to the conditions in *Petromyzon*. In the vicinity of this region where the substance enters the mesenchymatous tissue, there are many cells containing blue droplets. Another remarkable fact is the accumulation of many cells, apparently with amoeboid properties. They are easy to recognize on their large nucleus but the cytoplasm lacks blue stained material as far as can be seen.

The other *Pelobates*-specimen is essentially like the first one. The terminal part of the *filum terminale* is also somewhat widened forming an elongated terminal ventricle. In the hind part of this ventricle the fibre forms a distinct caudal mass that passes through the open terminal pore of the neural tube (fig. 15, 16). In the connective tissue the mass partly is split up like a brush of fine fibrillae. Most of these fine threads join again and form droplets, streaks and give the connective tissue a general smutched appearance. Even in this specimen many cells in this region contain alum haematoxylin positive matter.

It is naturally in this connection of great interest to examine the appearance of the caudal mass in an old larva with the tail during regressive metamorphosis. An old larva was therefore examined with a tail rudiment only 8 mm in length. The entire caudal part of the tail was in an advanced state of autolysis, Reissner's fibre had lost its smooth, thread-like shape for some distance and exhibited the same "spiny" appearance as the caudal masses of the younger larvae, described above. In this region of *filum terminale* many of the wall cells were decaying and even the normal ependymal cells were very irregularly arranged. In several places the fibre substance had passed between the ependymal cells. The amount of free cells in the central canal was much greater

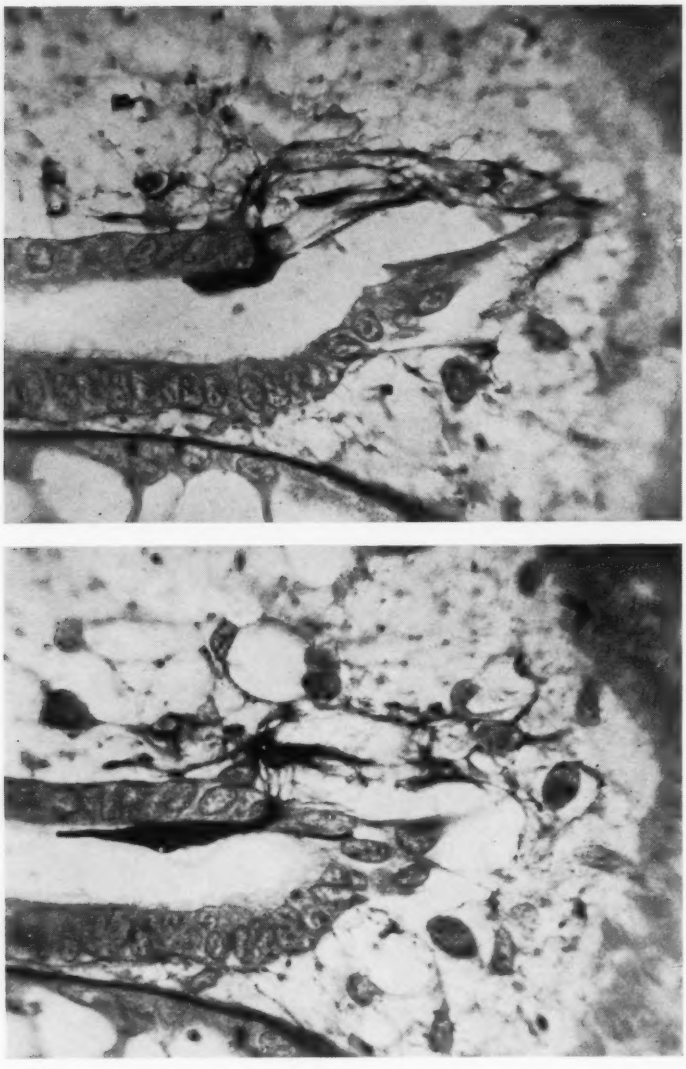

Fig. 15 and 16. *Pelobates*, 32 mm. Two adjacent sagittal sections through the end of the spinal cord. The caudal mass penetrates the wall and partly runs through the wide gap into the connective tissue, where it is spread over a wide area. Note cells with dark matter in the cytoplasm. Gomori's haematoxylin, 4 μ 600 x.

than usual. Some of these cells adhered to the fibre and most of them still seemed to be normal. The fibre could not be seen in the extreme end of the tail and even *filum terminale* was very ill-defined here and hardly recognizable.

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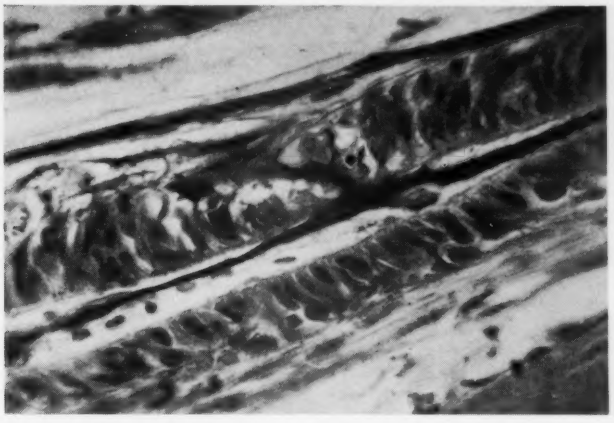

Fig. 17. *Xenopus*, adult. Caudal end of *filum terminale* (posterior end at right), with the caudal mass running into the glial interspaces. At the extreme left an enclosed cell can be seen in the fibre. Gomori's haematoxylin, 6 μ, 600 x.

ADULT *XENOPUS*

Caudally *filum terminale* has a Reissner's fibre as far as it is hollow. The caudalmost tip is solid and mainly consists of scattered ependymal cells surrounded by the meninges. It terminates in a small cluster of cells, probably the remains of the larval *filum terminale*.

The termination of Reissner's fibre is a large caudal mass, part of which passes between the ependymal wall cells and collects in the interspaces in the outer, glial layer of *filum terminale* (fig. 17). Some cells can also be found here that contain blue droplets in the cytoplasm.

In the caudal end of Reissner's fibre a few cells can be seen that apparently are totally enclosed in the fibre. The nuclei of these cells are large and pale. They have about the same width as the fibre but have a considerable length, the major axis being orientated in the same direction as the fibre. As their cell bodies are clearly delimited against the fibre substance and the cells are found only for a short distance in the caudal part and nowhere else, it seems reasonable not to interpret them as being normal components of the fibre. The general appearance of these cells and their capacity to adapt themselves to the shape of the fibre may suggest that they are some kind of amoeboid phagocytes. Free cells of the ependymal cell type are rather frequent in the central canal. Some of them adher to the fibre, but they are always easy to distinguish from the above-mentioned enclosed cells.

ANGUIS

KOLMER (1921) described a rather large terminal ventricle in young *Anguis*. A very delicate epithelium encloses this ventricle, which contains Reissner's fibre, degenerating cells and "Wandercellen."

In all the specimens that have been examined by the present author, the spinal cord terminates right behind the last pair of spinal nerves, without forming a *filum terminale*. Caudally the central canal forms the terminal ventricle described by KOLMER. However it has not been possible to discover an epithelial wall caudally, where the ventricle seems to be continuous with the interspaces of the very loose connective tissue in the extreme end of the tail.

The caudal portion of the central canal is slightly wider and Reissner's fibre usually thickens gradually. Where the central canal abruptly opens into the terminal ventricle, the fibre forms a caudal mass (fig. 18). This mass is a very conspicuous structure reaching caudally very far and giving off branches into the loose connective tissue. A transverse section of the caudal portion of the tail shows in the center several interspaces in the connective tissue that are direct continuations of the terminal ventricle. In several of them branches of the caudal mass are seen (fig. 19). Around this central portion several blood vessels are seen. On the whole the connective tissue is very richly vascularized. The only chrome haematoxylin matter, except the caudal mass, that is found in this region is the granular inclusion of some cells sometimes found in the vessels, probably granular leukocytes.

As described by KOLMER, free cells are usually found in the ventricle and they are often embedded in the caudal mass. They very seldom show any signs of degeneration and are probably free ependymal cells.

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DISCUSSION

Growth of the fibre in the central canal. It has been known for a long time that Reissner's fibre can grow in the central canal. DENDY (according to KOLMER) and others (NICHOLLS 1912 a, KOLMER 1921) showed that a fibre can be found even in the regenerated tails of lizards. KUX (1929) and MATHIS (1929) found that, in the cases when the neural tube in birds caudally opens through a pore, the fibre grows out through this pore and terminates freely in the amniotic fluid. The young stages of *Amphioxus* and *Xenopus* investigated for this paper also strongly support the view that the fibre is pushed backwards in the central canal, and reaches far behind the neural tube if given free passage. According to KOLMER (1921) the fibre is pushed caudad in the central canal by backward streams in the liquor, caused by ciliary action.

The formation of Reissner's fibre. Most investigators of later years seem to agree that the subcommissural organ is the place of formation of Reissner's fibre. According to JORDAN (1925), the cells of this organ give the same staining and chemical reactions as the fibre. Furthermore it now seems to be beyond any question that the organ, at least in part, functions as a gland. BARGMANN

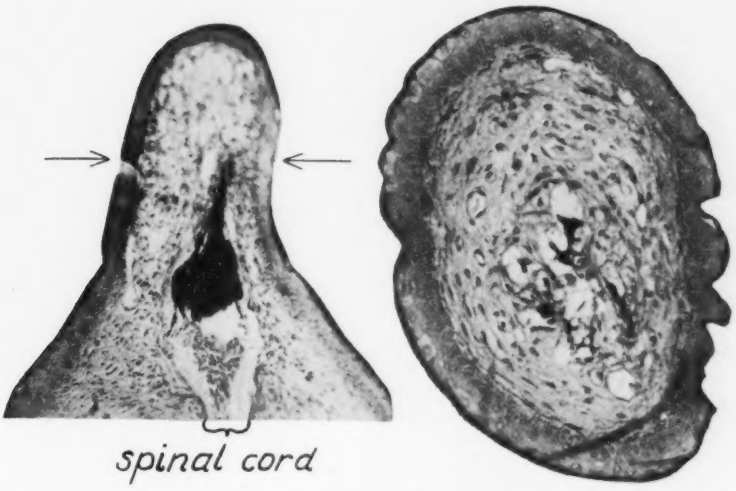

Fig. 18 and 19. *Anguis.* Left a horizontal section through the tail, with the caudally open spinal cord and a large mass, 7 μ, 104 x. Right a transverse section through about the same region as indicated by arrows in the left figure, 5 μ, 152 x. Several blood vessels and, in the centre, caudal mass branches. Gomori's haematoxylin.

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and SCHIEBLER (1952), however, did not consider that this secretion had any association to Reissner's fibre. According to WISLOCKI and LEDUC (1952, 1954), on the other hand, the subcommissural organ forms a secretion into the ventricle that is not immediately dissolved in the cerebrospinal fluid, but "condenses to form Reissner's fibre." According to WINGSTRAND (1953), the thread-like form of the fibre is due to the fact that the secretion is swept caudally by movements from strong cilia and in this manner forms the fibre that is pushed further caudad by liquor streams or movements of the ependymal cilia in the central canal. For further literature concerning the subcommissural organ, see the above-mentioned works.

The structure of Reissner's fibre. The statements of JORDAN (1919) and EBERL-ROTHE (1950, 1951) that the fibre is cellular, do not agree with the opinion that Reissner's fibre is a rod of secreted matter. The latter author succeeded in removing the fibre from fresh nervous systems of several mammals. In these isolated fibres she describes some elongated cells of a curious appearance. In sections she found cells in the caudal portion of the fibre even of some lower vertebrates.

Though all animals used for this study were carefully examined in this respect, it was never possible to trace a cell that could be considered as belonging to the fibre. Free cells and cell material in the central canal can often be seen adhering to the fibre, especially in the hind part, but it is generally easy to

see that these structures are irrelevant to the fibre. In one case only, really internal cells could be seen, namely the old *Xenopus* stage. In this fibre a few cells were found, all of them within a short region close to the caudal mass. The appearance of these cells indicates, however, that they are the result of a local invasion of phagocytes. The caudal mass often gives the impression of being a cellular structure, due to the fact that free cells are often totally surrounded by the caudal mass substance.

The cells in the fibre of *Pelobates,* described by EBERL-ROTHE, were twice as thick as the fibre itself, and therefore caused a considerable swelling of the fibre. If such cells always occur in Reissner's fibre, they must be very easy to find in the minute fibre of *Amphioxus* and especially in the often extremely tiny fibre of young animals. Although several such animals have been totally sectioned and the entire fibre carefully examined from the subcommissural organ to the caudal mass, such cells have never been seen. The Feulgen reaction was applied to the tail of one *Amphioxus* specimen but nowhere in the fibre could a staining reaction be seen. In a still unpublished electron-microscopical examination of Reissner's fibre, AFZELIUS and OLSSON confirm the opinion that the fibre is totally a homogeneous formation, as could be expected, if it is a string of secretion.

AGDUHR (1922) showed that the fibre, like a nerve process, seems to decrease in diameter in very old animals. He considers this fact as a proof that the fibre cannot be a secretion. If the fibre is formed from the subcommissural cells this statement as well proves to the contrary, viz. in case the organ either contains fewer producing cells in old animals, or the secretory action decreases with age.

The perpendicular threads. Several authors have observed the fine threads that often join Reissner's fibre and the walls of the central canal. KOLMER (1905) states that they probably are fused ependymal cilia, serving as supporting threads for Reissner's fibre. Even NICHOLLS (1912 a) mentions these "cilia-like processes" and considers that they stay and support the fibre. According to him, the fibre is modified protoplasm, as for instance hypertrophied cilia. He suggests that these fine threads form an integral part of Reissner's fibre in the same manner as long processes of the subcommissural organ form the rostral part of the fibre.

In small animals it is often very difficult to determine whether the fine threads are cilia or not. In large animals the difference between the two is often quite clear. Cilia usually are faintly red stained by phloxine, they are attached to intensely red basal corpuscles, they are short and rather thick. The fine threads are unstained or stained faintly blue by alum haematoxylin, they are never attached to basal corpuscles and vary in length and have always a minute thickness. Contrary to cilia they may be attached to the surface of the wall cells as well as between them. They may even adhere to coagulated mat-

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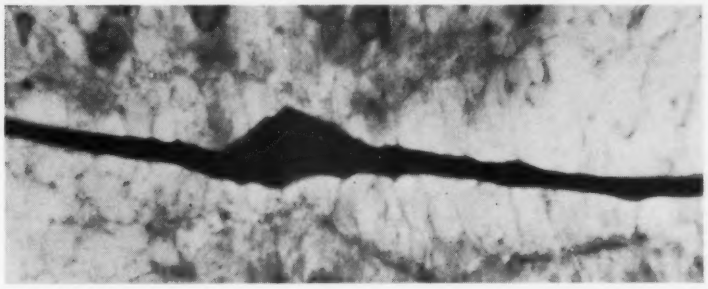

Fig. 20. *Myxine*. Detail of fig. 11. Note the perpendicular threads and the protrusions of the fibre where the threads begin. 870 x.

ter or cell remains in the central canal. To this, must be added the fact that these threads in most cases have a perpendicular position in relation to Reissner's fibre and that they sometimes anastomose. All this indicates that the threads are coagulated remains of a liquid.

In young *Squalus*, AGDUHR (1922) observed that the fibre sometimes is somewhat thickened in the places where it gives off the threads, "Verbindungsfäden." It is often seen, especially in the thick fibre of *Myxine* that the threads begin with a small protrusion of Reissner's fibre (fig. 20). In fact this condition is typical of the caudal mass, which sometimes may have the shape of an irregular star, each ray giving off a fine thread to the walls of the terminal ventricle. The threads always strongly give the impression of belonging to the mass or to the fibre.

If the threads are only parts of the fibre, it may be expected that they shall give the same staining reactions as the latter. They are, however, generally so slender that no staining can be traced at all, except a faint blue tint in the alum haematoxylin preparations. When the threads are thicker, as for instance where they fuse, they appear to stain in the same manner as the fibre itself.

The periodic acid-Schiff positive droplets (fig. 8) found in the central canal are of special interest in this connection. Except for Reissner's fibre and these droplets (and probably the perpendicular threads) there is no other material in the central canal, or its lining cell layers, that gives positive reaction whit this treatment. It seems reasonable to consider the droplets as a product derived from the fibre, either, already in the living specimen or as an artifact due to fixation. However it is of interest to note the possible existence in the central canal of free "fibre-substance" which may support the theory suggested by WISLOCKI and LEDUC (1952), that Reissner's fibre may be dissolved and release active material into the cerebrospinal fluid.

The caudal mass. According to KOLMER (1921) the fibre is renewed in the regenerated tail of a lizard owing to a temporary stimulation of the subcom-

missural organ, caused by the damaged tissues. However it is hard to believe in such occasional activity of the organ. At least in growing animals the fibre must always increase in length. That the growth of the fibre sometimes even exceeds that of the animal, is indicated at the *Xenopus* stages where the fibre reaches into the gut or neurenteric canal, and bird stages described by KUX (1929) and MATHIS (1929), where the fibre is seen in the amniotic fluid.

If Reissner's fibre is pushed backwards all the time, it is natural that there may be an accumulation of fibre substance in the hind part of animals with closed neural tubes. Such an accumulation, the caudal mass, has actually been found in most studied animals. In fact, the caudal mass strongly gives the impression of being a secretion, much more so than the fibre itself. This also will very well explain the contradictory statements of most authors, who have tried to interpret the mass as a regularly shaped structure.

NICHOLLS (1912 a, b, 1917) points out that the terminal portion of the highly elastic and taughtly stretched fibre, is an attachment plate. The normal condition is, according to him, that the fibre caudally is expanded to a trumpet-shaped structure which is inserted to the meningeal sheath. Only in a few cases, however, did NICHOLLS find this "terminal plug" in an undamaged condition, and his explanations for all the aberrant forms as artifacts often seem rather strained. In one of his papers (1912 a, plate 1, fig. 2) a tail of *Gasterosteus* is pictured, with a well developed "terminal plug." Judging by the photograph this better illustrates a fibre that gradually is transformed into a conical caudal mass, running into the connective tissue.

The most recent investigations of the caudal portion of Reissner's fibre, known to the present author, are the works of EBERL-ROTHE (1950, 1951). She states that the fibre caudally is hollow and the cavity caudally expanded. This elongated sac-like structure is often collapsed, which explains the irregular forms in sections. Also EBERL-ROTHE found several aberrant forms which cannot be explained in this way.

NICHOLLS (1912 b) and KOLMER (1921) have pointed out that autolysis starts in the fibre soon after death. In badly fixed material the fibre often looses its thread-like shape and becomes irregular. EBERL-ROTHE (1951) in some cases found that the fibre ended in "ein Gerinnsel", which was considered by her as a post-mortem phenomenon. The question then arises if the caudal mass always is an artifact.

It is possible that the perpendicular threads of the fibre and the mass are formed by the fixing fluid as a result of coagulation. But it is not probable that the mass itself is a result of post-mortem processes or the action of fixation. In many animals, as *Amphioxus,* ammocoetes, teleosts and tadpoles, the position of the mass is very superficial, as it is protected only by the skin, a thin layer of connective tissue and sometimes a single layer of ependymal cells. In such animals, fixed while alive, the fixation must have been effected in the

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region of the caudal mass long before it reached the fibre in the central canal. In the cases where the fibre is well preserved, there seems to be no reason to believe that the caudal mass has been influenced by post-mortem processes.

In the recently killed specimen, the fibre is almost a solid body that may be handled and extracted from the central canal (EBERL-ROTHE). Apparently the fibre deliquesces in the caudal part and then forms the caudal mass, that is permanently consumed by some unknown process. It is interesting to note in this connection that in a tadpole tail during regressive metamorphosis, where all tissues are in a condition of advanced autolysis, the fibre forms a giant caudal mass all over the region of resorption.

Reissner's fibre is often found in a coiled condition, which led NICHOLLS to believe that it is highly elastic. All cases of entangled, coiled or "snarled" fibres indicate, according to him, that the fibre has been injured somewhere in the central canal and retracted. JORDAN (1919) and EBERL-ROTHE (1951) are of a similar opinion. In my material I have often seen a coiled fibre in undamaged specimens that have been entirely sectioned, so the fibre could be observed from the subcommissural organ to the caudal mass. It is, however, not possible to determine if this slackened condition of the fibre exists already in life or is a result of fixation. Several authors have found that the fibre often is particularly caudally coiled (c.f. especially MATHIS 1935 and figures 1, 2 and 6). This may indicate that sometimes more of the fibre is pushed backwards than can be transformed into the viscous state.

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The fate of the caudal mass. Very little can be said about what happens to the caudal mass. Some authors have found remains of the fibre in the connective tissue near *ventriculus terminalis,* as SANDERS (1894), who states about the fibre that sometimes "it is attached to the surrounding tissue." The works of STUDNIČKA (1895, 1899) still seem to give the best idea on this subject. He supposes that the fibre eventually may be pushed backwards by the movements of the animal and then caudally break up into pieces or gradually dissolve in the surrounding connective tissue that is well supplied with lymphatic vessels. KOLMER (1921) declared that the statements that remains of the fibre may be found in the connective tissue are deceptive. This impression is caused by the degeneration of the caudalmost spinal cord cells.

The present author's results confirm entirely STUDNIČKA's views. The question of the fate of the mass in the connective tissue is still obscure. The mass certainly decomposes here and after this process the chrome alum haematoxylin-positive component does no longer show the same staining reactions. STUDNIČKA found several lymphatic vessels in this region and STERZI (1912) described lymphoid tissue round the end of the spinal cord in elasmobranchs. A notable concentration of vessels was seldom found in the material studied, but transport by body fluids is of course possible. In a few cases amoeboid cells

were found in this region and sometimes even cells containing chrome haematoxylin-positive droplets.

The development of Reissner's fibre. Both KOLMER (1918) and AGDUHR (1922) have pointed out that the fibre is developed earlier in lower vertebrates than in higher. With the aid of the staining methods according to GOMORI and GABE it has been possible in this investigation to find the first trace of Reissner's fibre in very young stages of all the examined animals. It is difficult to correlate developmental stages of different animals, but apparently there is no pronounced difference between the animals in this respect.

The examination of young stages has given the astonishing result that the fibre is often ill-defined or even imperceptible in the foremost part of the nervous system, although it easily can be seen in the hind part. This condition is found in all sufficiently young stages available, viz. in *Amphioxus, Petromyzon, Salmo* and *Xenopus.* A reasonable explanation of this phenomenon seems to be that the fibre in the beginning is a liquid that gradually condenses till it is transformed into a thick viscous state in the shape of a fibre. This condensation of the liquid into fibre-shape begins caudally and proceeds from behind and forward.

In the hind part of the neural tube of young *Petromyzon* larvae there is sometimes found a structure that without any doubt is primordium of a fibre (fig. 9). It gives the impression of being a semi-fluid substance covering the floor cells of the neural tube. At slightly older stages the substance has caudally condensed into a fibre. The 2 mm stages of *Xenopus* have apparently reached this stage. The fibre is clearly seen in the caudal end of the animal, but more craniad in the preparation the blue colour gradually fades and the fibre soon becomes invisible. In still older animals, the fibre may be seen all through the central canal, except in the brain cavities. The last part to appear in the old stages is the brush of fine threads (or membrane, according to EBERL-ROTHE) that joins the fibre and the subcommissural cells.

When examining the probable source in the brain, the subcommissural organ, the problem seems to be much more complicated. It is true that the subcommissural cells appear very early in development but the first discernible alum haematoxylin-positive substance is not found in them until in a considerably older stage. In *Xenopus,* the subcommissural cells could be identified at a 3 mm stage, but the first signs of positive-reacting substance in them did not show until at a 6 mm stage. Yet, the first trace of the fibre was observed in the caudal part of the animal already at the 2 mm stage. That alum haematoxylin-positive matter first appears distally is described also by DIEPEN, ENGELHARDT and SMITH-AGREDA (1954) in the hypothalamic hypophysial region. The first traces of such matter were found in the neurohypophysis and not until in older stages did the hypothalamic nuclei react positively. In my material are also

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found stages with a positive reacting subcommissural organ but a still incomplete fibre. This fact is not possible to explain as the result of the secretion turning alum haematoxylin-positive only caudally.

It is difficult to find an explanation for this strange condition, but there are some reasons for the following hypothesis. The very young embryos of *Xenopus* display a strong secretory action in the embryonic tissues. Even in the neural tube several cells are found producing granules. At these early stages the nervous system forms an elongated sac, closed in the anterior end while the posterior end communicates with the gut through *canalis neurentericus*. Probably there is an outflow of liquid from the neural tube, through the neurenteric canal, into the gut. The large accumulation of granules and non-granulated alum haematoxylin-positive matter (fig. 14), which is always found at the point where the neurenteric canal suddenly opens into the gut, may well confirm this opinion.

Later on the secretion gradually condenses in the hind part and forms a viscous liquid running above the floor cells of the neural tube. At older stages the condensing process proceeds and the almost solid Reissner's fibre is formed. Accordingly, the fibre grows in about the same manner as a stalagmite. When the brain reaches a higher degree of differentiation, the production of fibre-forming secretion is restricted to a small region, *organon subcommissuralis* in vertebrates and *organon infundibularis* in *Amphioxus*.

The single *Amphioxus*-specimen where there is a fibre only in the anterior half of the central canal is an exception, possibly the result of an accidental breakage of the fibre. The blue stained material in the terminal ventricle should then be the only remains of the caudal part of the original fibre.

General remarks about filum terminale. Reissner's fibre evidently tends to reach the extreme end of the cavity in which it is situated. Even the central canal apparently has a tendency towards reaching as far back as possible (fig. 21). In animals where no roots are needed in the caudal portion of the spinal cord, the central canal may continue caudad as a non-nervous *filum terminale*. The termination of this structure is often widened, the central canal then forming a *ventriculus terminalis*. In some cases this ventricle almost seems to be a storing place for the caudal mass (*Amphioxus, Petromyzon,* frog tadpoles). In all animals examined, except *Amphioxus* and young specimes of vertebrates, the terminal ventricle opens through a "pore," that in most cases is directed dorsad. The same condition is also described in mammals (horse, VERMEULEN 1916).

It is possible that in the future, when more knowledge about these structures has been collected, the existence of a *filum terminale* may be connected with the function of the Reissner's fibre apparatus.

Fig. 21. *Lebistes,* 8.0 mm. Median, sagittal section through the tail showing, between the hypuralia and the last dorsal spine, the long *filum terminale* in the extreme end of which the caudal mass is situated. *Filum terminale* is caudally a single cell lined tube with a central canal wide enough for Reissner's fibre. * indicates spinal cord. Gomori's haematoxylin. 5 μ, 100 x.

SUMMARY

Reissner's fibre is an extremely early structure in the ontogenesis. The first signs of a fibre appear in the caudal portion of the neural tube and not until at considerably older stages, a distinct subcommissural organ and the anterior portion of the fibre may be discerned. Apparently the fibre is permanently pushed backwards, and in young animals where the neurenteric canal is still open, the termination of the fibre may be found in the gut. At older stages the fibre substance usually accumulates in the caudal part of the spinal cord as a caudal mass that seems to have less viscosity than the fibre itself. Part of this mass often leaves the spinal cord either through a pore or between the wall cells, into the surrounding tissues, where it apparently is disposed of in some way.

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SKELETON OF CYPRINOID FISHES IN RELATION TO PHYLOGENETIC STUDIES: 7. THE SKULL AND WEBERIAN APPARATUS OF CYPRININAE (*CYPRINIDAE*)

BY

L. S. RAMASWAMI

(Department of Zoology, University of Mysore, Central College, Bangalore.)

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INTRODUCTION

Having described the skull and weberian apparatus in the subfamily Gobioninae (Cyprinidae) (RAMASWAMI, '54), I now venture to place on record the same in the subfamily Cyprininae. This subfamily comprises the largest number of fresh-water genera, represented in America, Eurasia and Africa. Of these, as REGAN ('11) remarked, the greatest variety is found in Asia. Günther (1868) split the family into a number of groups and Weber and de BEAUFORT ('16) put the Indo-Australian cyprinids under three subfamilies. BOULENGER ('22) included the Catostomidae, Homalopteridae and Cobitidae under the Cyprinidae and altogether 14 subfamilies are recognized (SEDGWICK, '05). At the time REGAN ('11) described the genera, he did not recognize subfamilies of the Cyprinidae as he thought that the characters exhibited by them were not well marked for creating subfamilies but put them in different groups. He, however, stated that GÜNTHER's (1868) Cyprinina appeared to be a natural group after excluding the North American forms and adding a few others. NICHOLS ('38) divided the Cyprinidae into 8 subfamilies, basing his classification mostly on external characters. As recently as 1940, Berg considered the Cyprinidae as comprising only four subfamilies: Cyprinini, Psilorhynchini, Gobiobotini and Hypophthalmichthyini. It may just suffice to mention here

that *Psilorhynchus* has already been elevated as the type genus of the family Psilorhynchidae (Hora, '25; Ramaswami, '52 b); *Gobiobotia*, though exhibiting specialised characters, could still be treated as a member of Gobioninae but distinct from others and it is not therefore necessary to create another subfamily to accommodate it (Ramaswami, '55). Berg ('40), however, included a number of gudgeons under Cyprinini, which have now been included under Gobioninae. Obviously Berg did not see the propriety of splitting his Cyprinini examples as has been done by previous systematists and put them under new subfamilies.

The following genera of Cyprininae (= Cyprinini Berg, '40) have been examined by me with a view to study their interrelationships and those species which are followed by the letters 'BM', were examined at the British Museum of Natural History, London, and those with 'SU', at the Natural History Museum, Stanford University, Stanford, Calif.

1. *Amblypharyngodon mola* (Ham. Buch.)
2. *Aphyocypris normalis* Nichols (*Yaoshanicus normalis* [Nichols and Pope])
3. *Aristichthys nobilis* (Richards)
4. *Aspidoparia morar* (Ham.)
5. *Aspius spilurus* Günther
6. *Barbus* (*Tor*) *khudree* Sykes; *B. pinnarautus* Day; *B. sarana* (Ham. Buch.)
7. *Barilius gatensis* (C. & V.)
8. *Capoeta fundulus* Günth., BM.
9. *Carassius auratus* (L.)
10. *Catla catla* (Ham.)
11. *Cirrhina mrigala* (Ham. Buch.); *C. reba* (Ham. Buch.)
12. *Chela bacaila* (Ham. Buch.)
13. *Crossochilus barbatulus* Heck., BM.; *C. latius* (Ham.)
14. *Cyprinus carpio* (L.)
15. *Danio equipinnatus* (McClelland)
16. *Diptychus maculatus* Stein.
17. *Eremichthys acros* Hubbs & Miller
18. *Esomus barbatus* (Jerd.)
19. *Garra lamta* (Ham. Buch.), BM; *G. mullya* (Sykes); *G. quadrimaculatus* (Rupp.), BM; *G. variabilis* Heck., BM.
20. *Hyborhynchus notatus* Raf.
21. *Labeo boggutt* (Sykes); *L. dero* (Ham.); *L. diplostomus* (Heck.), BM; *L. dyocheilus* (McClelland) *L. horie* (Cuv.), BM; *L. niloticus* Forsk., BM; *L. rohita* (Ham. Buch.)
22. *Leuciscus leuciscus*
23. *Moapa coriacea* Hubbs & Miller

24. *Mystacoleucus ogilbii* Sykes
25. *Notropis cornutus*
26. *Opsariichthys bidens* Günther, SU.
27. *Orienus sinuatus* (Heck.)
28. *Parapsilorhynchus tentaculatus* Annandale
29. *Phoxinus phoxinus* L.
30. *Ptychidio jordani* Myers, SU.
31. *Ptychocheilus oreganensis* (*Leuciscus oreganensis* [Richards]), SU
32. *Puntungia* sp., SU.
33. *Rasbora caverii* (Jerd.)
34. *Scaphiodon baluchiorum* Jenkins
35. *Scardinius erythrophthalmus* (*Leuciscus erythrophthalmus* [L.])
36. *Schizothorax labiatus* (McClelland)
37. *Thynnichthys sandkhol* (Sykes)
38. *Tylognathus elegans* Günth., BM; *T. klatti* Kosswig, BM; *T. nanus* Heck., BM; *T. steinitziorum* Kosswig, BM.
39. *Varicorhinus beso* Rüppel, BM.

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For purposes of comparison, I have also studied the osteology of *Catostomus camersonii camersonii* (Lacep.) (Catostomidae), considered to be a generalised representative of the family and of *Hypophthalmichthys* (Hypophthalmichthyini, Cyprinidae).

OBSERVATIONS

In the Cyprinidae, the ethmoid (*mesethmoid:* Goodrich, '09; Regan, '11; Sarbahi, '33; Girgis, '52; *dermethmoid*: Gregory, '33) is noticed in two parts; an extensive dorsal part, — the supraethmoid (de Beer, '37) which is posteriorly in contact with the frontals, and an anteroventral portion. The dorsal supraethmoid part may extend laterally somuch that it may be visible on the ventral aspect of the skull also as in *Labeo* (*niloticus* and *diplostomus*, figs. 5 and 6, se), *Rasbora* (fig. 7, se), *Esomus* (fig. 8, se), *Varicorhinus* (fig. 10, se), *Danio, Chela, Crossochilus, Diptychus, Aspidoparia, Amblypharyngodon, Scaphiodon, Leuciscus, Aristichthys, Ptychidio, Notropis, Tylognathus, Garra, Orienus, Schizothorax, Cirrhina* and *Barbus*. The dorsal supraethmoid portion may be narrow as in *Barilius* and may show a deep indentation in which the median rostral rests. The median projections of the supraethmoid seen in some Gobioninae (Ramaswami, '55) are not noticed in the Cyprinidae. In *Aristichthys* between the supraethmoid and the frontals, there is a small median fontanel; I do not know if this is an individual variation, as normally, there is no fontanel in this region of the skull of Cyprininae.

The anteroventral part of the ethmoid is tipped with a cartilaginous epiphysis in the Cyprininae, in which an ossification is noticed. This has been called the preethmoid bone. The preethmoid may articulate with the ethmoid only as in *Labeo diplostomus* (fig. 6, pre), *Barbus, Mystacoleucus, Opsariichthys, Aspius, Hyborhynchus, Moapa, Eremichthys* and *Danio* or with the prevomer also as in *Rasbora* (fig. 7, pre), *Varicorhinus* (fig. 10, pre), *Garra, Oreinus, Schizothorax, Cirrhina, Barilius, Chela, Crossochilus, Aspidoparia, Diptychus, Tylognathus, Aphyocypris, Ptychidio* and *Scaphiodon*. However, in the figure of *Labeo* (species?) GREGORY ('33) delineated the preethmoid as connecting both ethmoid and prevomer. SARBAHI ('33) did not depict a preethmoid in his figure of the skull of *Labeo rohita*, but in his figure 14 (p. 315), there is intercalated between the palatine and the socalled mesethmoid a nodule which is not labelled and which answers to a preethmoid. It appears clear that SARBAHI did not have access to the important paper of STARKS ('26) where this bone has been described in great detail. GIRGIS ('52) shows a preethmoid in *Labeo horie*.

Among the forms described by STARKS ('26) the preethmoid is a projection on either side of the prevomerine process giving articulation to the 'submaxillary' (see below) and the palatine; sometimes, there may be two distinct processes of the preethmoid for these two articulations. In the 'spiny-rayed' fishes, according to him, there may be an articulating surface representing a 'preethmoid' formed by the prefrontal (= lateral ethmoid) and the prevomer or from the prevomer and mesethmoid (= ethmoid).

A preethmoid is completely wanting in *Esomus* (figs. 3 and 8), *Leuciscus, Phoxinus, Scardinius, Aristichthys* and *Notropis*; therefore, these genera stand apart from their congeners in not possessing this bone. It is not clear why a bone so commonly noticed among the Cyprinidae should be absent in a few genera of the examined species.

The term 'submaxillary' was used by SAGEMEHL (1884, 1885, 1891) for describing a pair (or pairs) of bones in the ethmoid region spanning the maxilla anteriorly and prevomer or palatine behind; GREGORY ('33) considered these to be in the nature of sesamoid ossifications. REGAN ('11) described in the Cypriniformes, the anterolateral bony epiphysis of the prevomer as 'septomaxillary' (= preethmoid) and one or two pairs of cartilages (Catostomidae) or bones (Cobitidae) connecting the 'septomaxilla' and palatine with the maxilla as the 'submaxillary' or prepalatine cartilage or bones. I have called elsewhere (RAMASWAMI, '55) the cartilage between the preethmoid and the maxilla, a second preethmoid. In the majority of Cyprininae, it is a very thin cartilage; however, in *Cyprinus* and *Amblyrhynchichthys* it undergoes ossification according to SAGEMEHL (1891); I have also noticed an ossified second preethmoid in *Cyprinus*.

The prevomer in the Cyprininae extends anteriorly to the ethmoid on the

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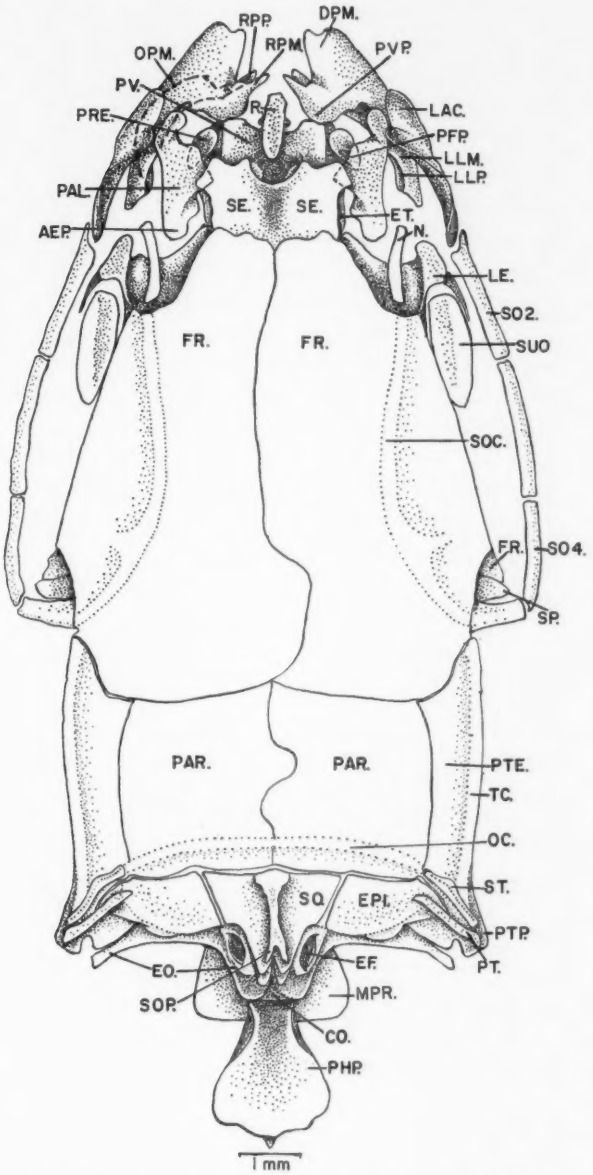

1. Dorsal view of the skull of *Labeo boggutt* (Sykes).

ventral aspect. This is noticed in *Labeo* (figs. 1, 4, 5 and 6, pv), *Rasbora* (figs. 2 and 7, pv), *Esomus* (figs. 3 and 8, pv), *Garra* (fig. 9, pv), *Cirrhina*, *Barbus*, *Mystacoleucus*, etc.; in *Orienus* and *Schizothorax* the extension is

very little. In *Amblypharyngodon* the prevomer extends in front of the ethmoid in the form of a horizontal hour-glass and its lateral processes are not associated with the preethmoids as these are absent. In *Chela* the prevomer does not extend beyond the ethmoid anteriorly. The posterior process which underlies the parasphenoid may completely cover the breadth of it in this region as in *Orienus* and *Schizothorax*. The posterior process of the prevomer is short and broad in *Labeo niloticus* (fig. 5), *Rasbora* (fig. 7), *Garra* (fig. 9), *Aphyocypris, Aristichthys, Ptychidio* and *Danio*; in *L. boggutt* (fig. 4), *L. diplostomus* (fig. 6) *Esomus* (fig. 8), *Cirrhina, Barbus, Aspidoparia, Diptychus, Scaphiodon, Mystacoleucus, Crossochilus, Moapa, Eremichthys, Hyborhynchus* and *Notropis*, the process is comparatively longer.

In *Labeo rohita*, SARBAHI ('33) has erroneously depicted the prevomer as almost a rectangular bone. In my alizarin preparations, two lateral limbs could be clearly made out and the mesial part underlies the anterior tip of the parasphenoid. Moreover, the median part is depressed. In *Barilius, Chela, Opsariichthys, Aspius* and *Amblypharyngodon* the two posterior (or lateral) limbs of the prevomer exhibit a deep depression between them as in *Labeo*. In fact in *Amblypharyngodon* the anterior extension of the parasphenoid is exposed and not overlain by the prevomer as in other examples.

According to REGAN ('11) in the Cyprinidae, the premaxillaries exclude the maxillae from the gape of the mouth. This is true of all the genera examined by me.

Each premaxilla has generally a dorsal limb directed towards the median rostral and the lateral limb is disposed ventrally to the maxilla, thus keeping the latter out of the gape of the mouth. This is seen in *Barbus, Schizothorax, Rasbora* (figs. 2 and 7, rpp), *Esomus* (figs. 3 and 8, rpp), *Mystacoleucus, Barilius, Chela, Crossochilus* etc. In *Labeo, Garra, Orienus, Cirrhina* and *Scaphiodon* the rostral limb is very short and it is wanting in *Diptychus* and *Ptychidio*. In *Diptychus* the mesial end of the ventral limb is serrated. In *Danio* the rostral limb is short and broad and ends in front of the laterally expanded rostral; in *Amblypharyngodon* the short and broad limbs of either side approximate and cover the anterior portion of the median rostral.

The maxillae in the Cyprininae show as many as five processes each. From the mesial end there is a blunt or pointed premaxillary process (figs. 2 and 7, dpm) dorsally for the insertion of a ligament from the palatine, and a pointed ventral rostral process (rpm), holding as it were the premaxilla in a fork. This forklike arrangement is lost in *Labeo* (figs. 1, 4, 5, and 6), *Esomus* (figs. 3 and 8), *Garra* (fig. 9) and *Varicorhinus* (fig. 10) as the two processes are apart. The lateral portion of the maxilla arches over the premaxilla and the horizontal limb may show rounded articular facets towards the preethmoid (figs. 9 and 10, prm) and the palatine (ppm); in *Rasbora* (figs. 2 and 7, ppp) there is only one process which articulates with the preethmoid and prevomer;

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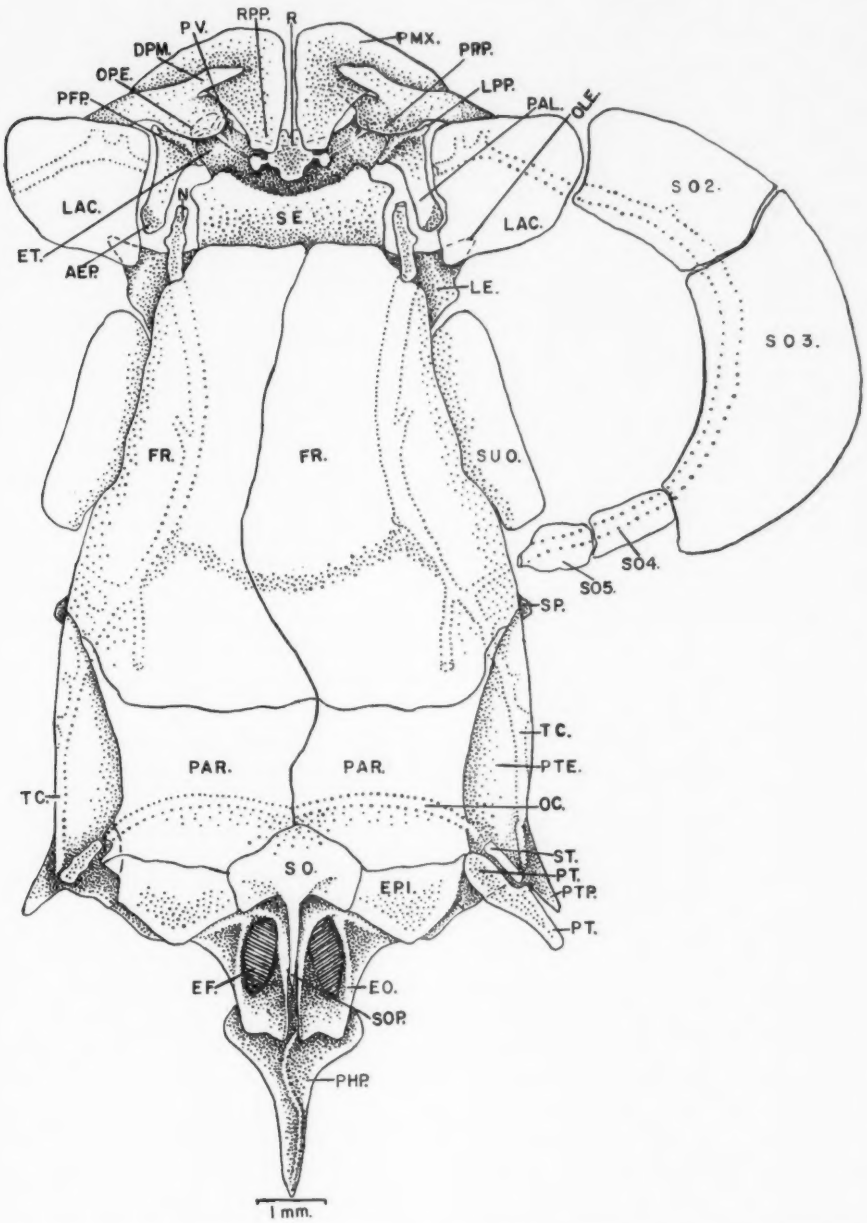

2. Dorsal view of the skull of *Rasbora caverii* (Jerd.); the posttemporal and suborbitals shown on one side.

in *Labeo* there are two, and the mesial one (figs. 1, 4, 5 and 6, pvp) articulates with the prevomer; in *Esomus* (figs. 3 and 8, pvp) the only process contacts the prevomer. From the anterior face of the maxilla, there is generally a large or small articular area (fig. 8, pam) for the attachment of a ligament of the adductor mandibulae muscles. The lateral limb of the maxilla projects dorsally to the similar limb of the premaxilla. In *L. rohita* the ventral rostral process is short and blunt and in *Barbus sarana, Mystacoleucus, Danio* and *Chela* the process for the attachment of the ligament (of the adductor mand. muscles) is shorter and the dorsal premaxillary processes meet mesially above the rostral processes of the premaxilla. In *Cirrhina* the rostral process of the maxilla is absent and the lateral limb is shorter than that of the premaxilla. In *Amblypharyngodon* the dorsal process directed towards the premaxilla is inconspicuous.

The lateral ethmoid (prefrontal *autt.*) separates the anterior nasal region from the posterior orbital region. The extension of the bone laterally is in contact with the suborbital sensory canal bone and shows generally an anterior and a posterior process (see below). Mesially the bone shows contact with the frontal dorsally, and ventrally with the ethmoid, prevomer, parasphenoid and orbitasphenoid. Great variation is noticed in the processes arising from the lateral edge of the bone. In a large number of cyprinines examined (*Rasbora* [figs. 2 and 7, le], *Esomus* [figs. 3 and 8, le], *Garra* [fig. 9], *Varicorhinus* [fig. 10], *Barilius* etc.) both processes are poorly represented; in *Cirrhina* the processes are better developed and in some species of *Labeo* (figs. 4 and 6, lp) and *Mystacoleucus* the anterior process is longer. In *Scaphiodon, Diptychus, Aspidoparia, Amblypharyngodon, Aspius, Ptychidio, Aristichthys, Aphyocypris, Phoxinus, Leuciscus, Moapa. Eremichthys, Hyborhynchus* and *Notropis,* the processes are wanting.

The palatine is short in the Cyprininae and shows anteriorly a facet (figs. 1, 2, 4, 8, 9 and 10, pfp) to articulate with the rounded epiphysis-like preethmoid when present, or otherwise with the cartilaginous epiphysis as in *Esomus.* It has also a rounded head posteriorly to fit into the facet of the entopterygoid (figs. 1—3, 6—10, aep). From the beak-like prominence of the palatine in the region of the palato-ethmoid articulation, there arises a ligament which is inserted into the median ethmoid projection. There is also a ligament arising from the anterior end of the palatine to be inserted into the dorsal premaxillary process of the maxilla. In examples like *Esomus* (fig. 8, lpp), *Cirrhina, Aspidoparia, Eremichthys, Aristichthys* and *Aspius* the palatine also shows laterally a facet for the articulation with the lacrimal. In *Leuciscus, Phoxinus, Aphyocypris, Ptychidio, Moapa, Hyborhynchus* and *Notropis,* the lateral lacrimal process is absent. In *Amblypharyngodon* the palatine is a rectangular bone and from its anterior end an anteromesial process is attached to the prevomerine process by a ligament and similarly, the

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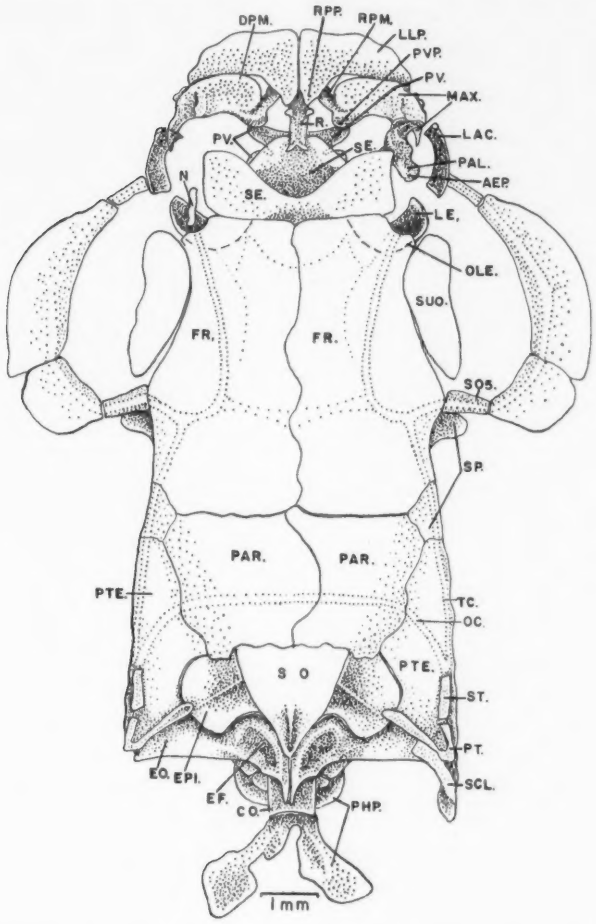

3. Dorsal view of the skull of *Esomus barbatus* (Jerd.); lacrimals gently pulled to show structures; supracleithrum shown on one side.

posteromesial process is connected with the ethmoid by a ligament. Dorsally there is a facet for articulation with the maxilla.

The rostral (figs. 1—10, r) is a median bone. It is considered to be a specialised part of the ethmoid (GOODRICH, '09). Generally the median rostral is cylindrical, disposed obliquely dorsoventrally with the two ends rounded and the middle part showing two articular processes more dorsally. Sometimes these processes are absent. Numerous modifications of this arrangement are noticed, and in *Danio*, the bone extends horizontally with a short median limb looking like the letter Y. The dorsal end of the rostral is attached to the

two rostral processes of the premaxillae by a pair of thick ligaments; the ventral end is attached by a ligament with the anterior portion of the ethmoid. A ligament from the articular processes of the rostral proceeds to the antero-ventral limbs of the maxillae.

The orbitotemporal region: The frontals (figs. 1—3, fr) form the roof anteriorly and the parietals (pa) posteriorly; it has already been noted that between the supraethmoid and the frontals, a small fontanel is noticed in *Aristichthys*. The occipital sensory canal runs through the parietals either at their posterior border or slightly anteriorly (*Esomus*) or far anteriorly as in *Chela*, making a big arch.

The supraorbitals may be large, extending posteriorly as far as the suborbital or sphenotic (*Rasbora* [figs. 2 and 7, suo], *Esomus* [figs. 1, 4 and 5, suo], *Aphyocypris*); they are short and broad in *Ptychidio*; in *Labeo* (figs. 1, 4 and 5, suo) and *Garra* (fig. 9, suo) they are short. The suborbitals form a chain of 5—6 bones carrying the suborbital sensory canal, the first one being called a lacrimal (figs. 1—3, 5, 7 and 8, lac). The ossicles may be broad as in *Rasbora* (figs. 2 and 7, so2—so5), *Ptychidio* and *Cyprinus* or they may be in the form of tubular ossicles (fig. 5, so2—so5).

The orbitosphenoids are usually paired. However, in *Cyprinus* and *Carassius*, the paired bones have united mesially to form a single bone. Such a feature is a general character of the Cobitidae (Ramaswami, '53). Sometimes the two orbitosphenoids send mesially a small projection towards the parasphenoid and may meet a similar projection from the parasphenoid thus forming an interorbital septum (see fig. 11.). This feature is noticed in *Labeo, Crossochilus, Barbus, Mystacoleucus* and *Chela*; in others the two orbitosphenoids may meet the parasphenoid dorsally and thus separate the two orbits as in *Rasbora* (fig. 7, os), *Esomus* (fig. 8, os), *Garra* (fig. 9, os), *Orienus, Schizothorax, Cirrhina, Barilius, Danio, Leuciscus, Phoxinus, Moapa, Eremichthys, Hyborhynchus* and *Notropis*.

The pleurosphenoids (alisphenoid *autt.*) (figs. 4, 5, 7 and 9, pls) form the posterior wall in the orbitotemporal region. The optic foramen (of) is situated between the parasphenoid, pleurosphenoid and orbitosphenoid. The pleurosphenoid may bear a deep groove in it as in *Garra* (fig. 9, gv) for the passage of a blood vessel. Posteriorly, in all the forms, the bone comes in contact with the parasphenoid and prootic leaving openings for the myodome (figs. 7, 8 and 9, myo) and trigeminofacial nerves.

The anterior extension of the parasphenoid (figs. 4—11, pas) does not show any variation; it is underlain by the prevomer (pv) in the ethmoid region. The long or short posterior prevomerine process (fig. 4, ppv), when present, runs ventrally to the parasphenoid. It has already been noted that in some forms (*Crossochilus, Labeo, Barbus*) the parasphenoid sends dorsally a short process which comes in contact with a similar median process formed

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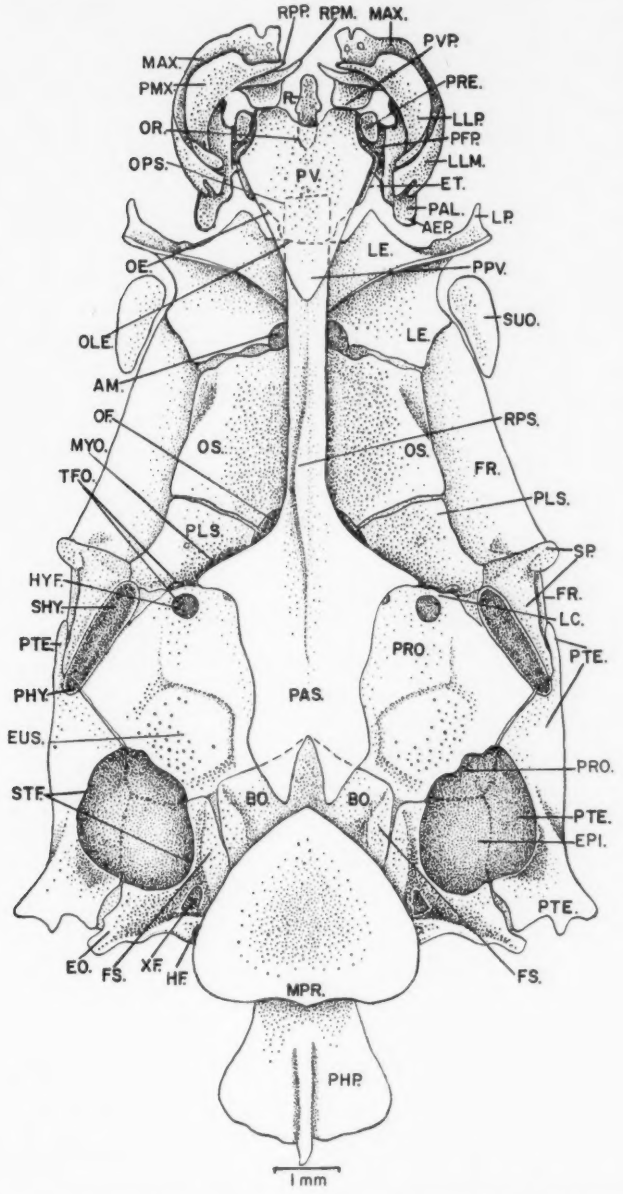

4. Ventral view of the skull of *Labeo boggutt* (Sykes); suborbitals not shown.

by the orbitosphenoids, forming an interorbital septum. At the region of the prootics, the parasphenoid sends lateral processes and broadens out. It forms the floor of the posterior myodome (fig. 11, pmy) and extends ventrally to

the basioccipital. Invariably the parasphenoid shows posteriorly a forked appearance.

The myodome: I shall describe the disposition of the myodome or the eye-muscle canal in *L. boggutt* whose longitudinal sectional view has been drawn. In the allied species *Rohu*, SARBAHI ('33, p. 323) described the myodome as follows: "In *Rohu* the myodome in the dry skull is a large median space lying between the floor of the cranial cavity above and the parasphenoid below. It is bounded posteriorly by the prootic and the basioccipital, dorsally by the alisphenoid, ventrally by the parasphenoid and anteriorly by the orbitosphenoid." In *boggutt* (fig. 11), the entrance of the myodome is a large opening (omy) bound laterally and dorsally by prootic (pro) and mesially and ventrally by the parasphenoid (pas); the canal is a spacious chamber (pm) posteriorly bounded by and ending in (pm') the basioccipital (bo). Anteriorly the prootic (laterally and dorsally) and parasphenoid (ventrally) bound it. The pleurosphenoid (pls) (alisphenoid according to SARBAHI) does not take part in it and the orbitosphenoid (os) is far anteriorly situated.

SARBAHI ('33) does not refer to an anterior myodome. According to GOODRICH ('30) the anterior myodome is hollowed out in the ethmoid region by the oblique muscles. In the Cyprininae studied by me, the entry of the oblique muscles could be seen through an opening (fig. 4, am) between the parasphenoid and the lateral ethmoid and the muscles are actually inserted on the ventral aspect of the ethmoid.

The auditory region: The auditory region of the cyprinids is formed by the sphenotics (figs. 4, 5, 7, 8, 9 and 11, sp) and pterotics (pte) on the sides, the prootics (pro) ventrally and the epiotics (epi) dorsally (see below: opisthotic); the supraoccipital (so), basioccipital (bo) and the exoccipitals (eo) form the posteromesial boundary of the skull, while the parietals (fig. 11, par) roof the skull in the pterotic region.

The sphenotic forms the anterolateral boundary of the auditory region and accommodates the anterior semicircular canal (fig. 11, uoa, loa) as could be seen in a longitudinal sectional view. The bone projects laterally as a spinous or broad ledge at the base of which, ventrally, there is a large depression or fossa extending into the pterotic behind; the anterior facet of the hyomandibula articulates in this fossa (figs. 4, 5, 7, 8 and 9, shy) of the sphenotic.

Generally the sphenotic is not noticed dorsally as a roofing bone and does not accommodate the temporal sensory canal proceeding from the frontal into the pterotic. This happens because the posterior portion of the frontal meets the pterotic (fig. 1) and the temporal canal runs directly from the frontal into pterotic. In *Esomus* (fig. 3) however, the sphenotic (sp) is noticed as a triangular roofing bone laterally to the frontal (fr) and parietal (par) and anteriorly to the pterotic (pte); ventrally to the frontal, the sphenotic extends anteriorly to meet the pleurosphenoid and a lateral blunt projection of

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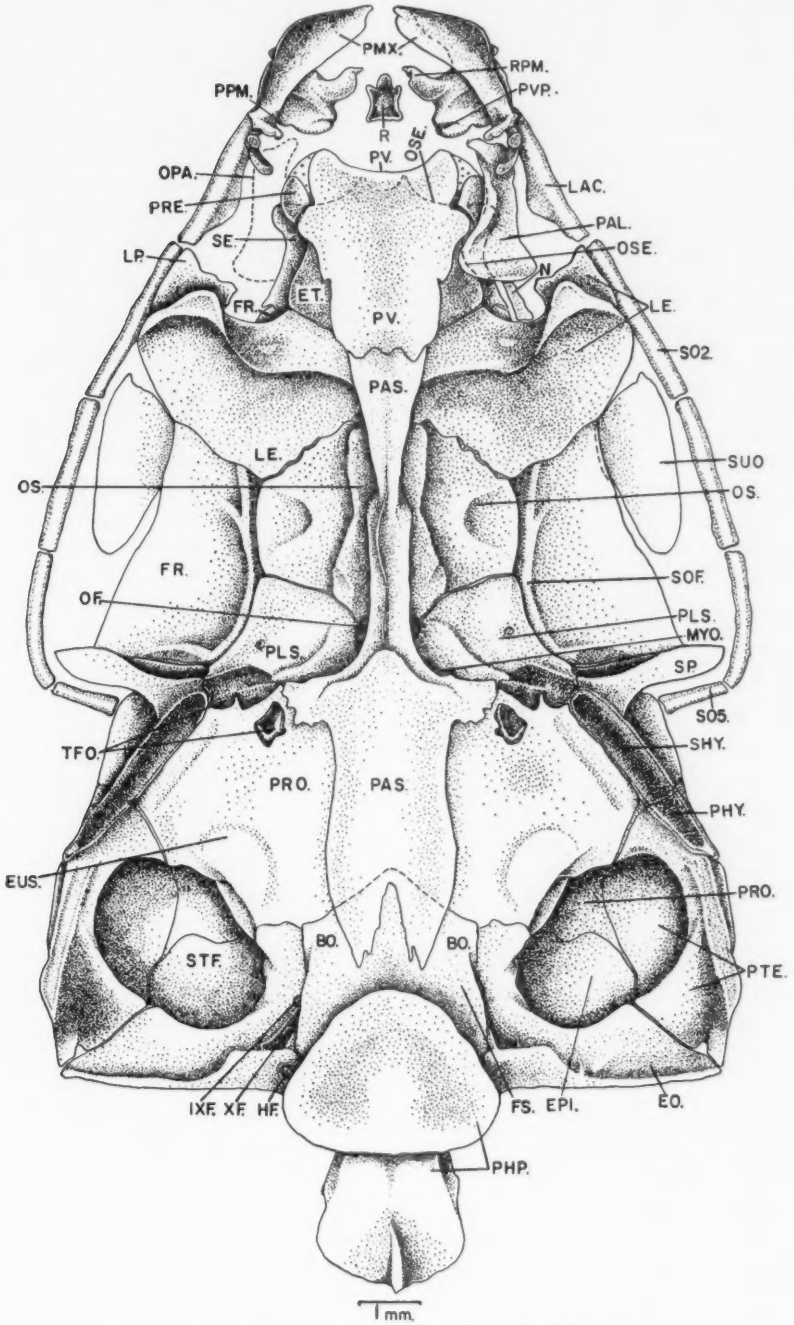
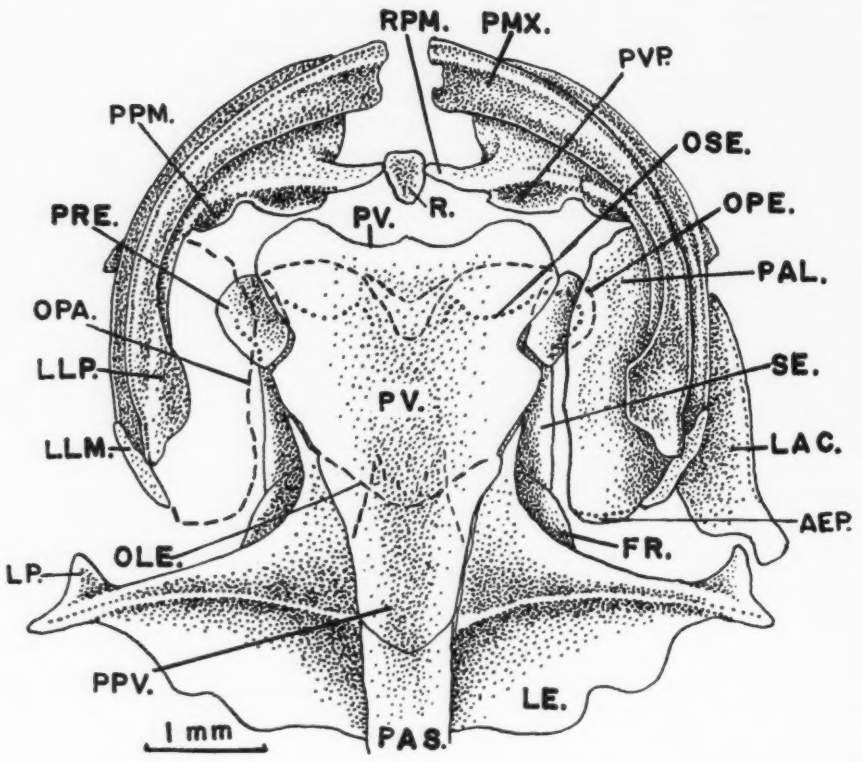


5. Ventral view of the skull of *Labeo niloticus* Forsk.



6. Ventral view of the ethmoid region of *Labeo diplostomus* (Heck.).

it could be seen in the dorsal aspect. This is the only genus of the examined cyprinids where such a condition is met with.

Ventrally between the parasphenoid and sphenotic-pterotic and posterior to the pleurosphenoid (figs. 4, 5, 7, 8 and 9, pls) is the large prootic (pro) in which the trigeminofacial nerve foramina are noticed. The bony border in front of the facial foramen (fig. 4, hyf) is the lateral commissure (lc). Between the prootic and the parasphenoid, there is a large foramen (figs. 7 and 9, hya) for the hypophysial artery. The bone also forms the anteromesial boundary of the subtemporal fossa (stf). In the longitudinal sectional view, it is noticed that this bone (fig. 11, pro) forms a large part of the otic capsule wall posteriorly to the sphenotic (sp) and laterally to the parasphenoid (pas), accommodating largely the utriculus (cu) of the membranous labyrinth. In the ventral aspect of the skulls examined, towards the subtemporal fossa an enlargement of the prootic (figs. 4 and 5, eus) is noticed; this is the utricular bulge.

The pterotic (figs. 4, 5, 7, 8 and 9, pte) forms the posterolateral boundary

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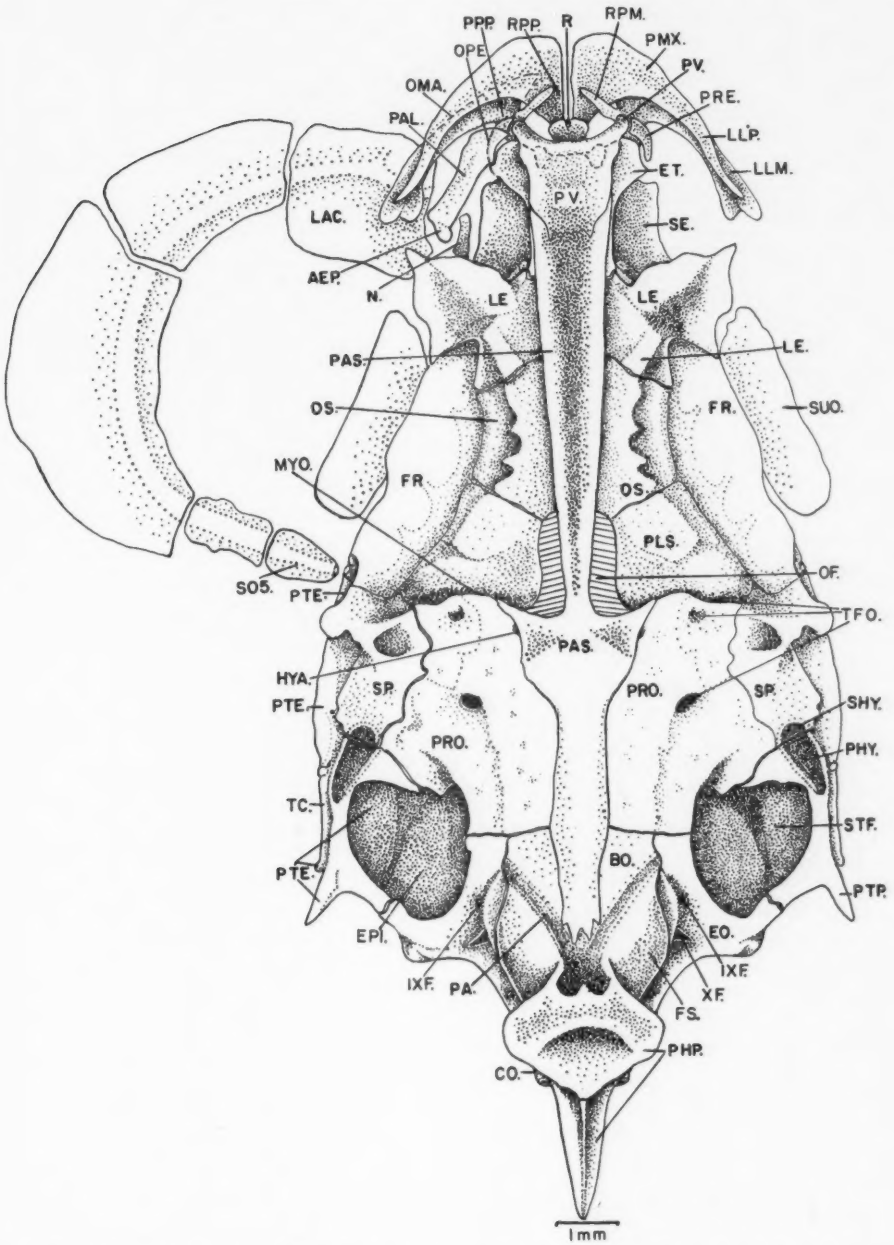
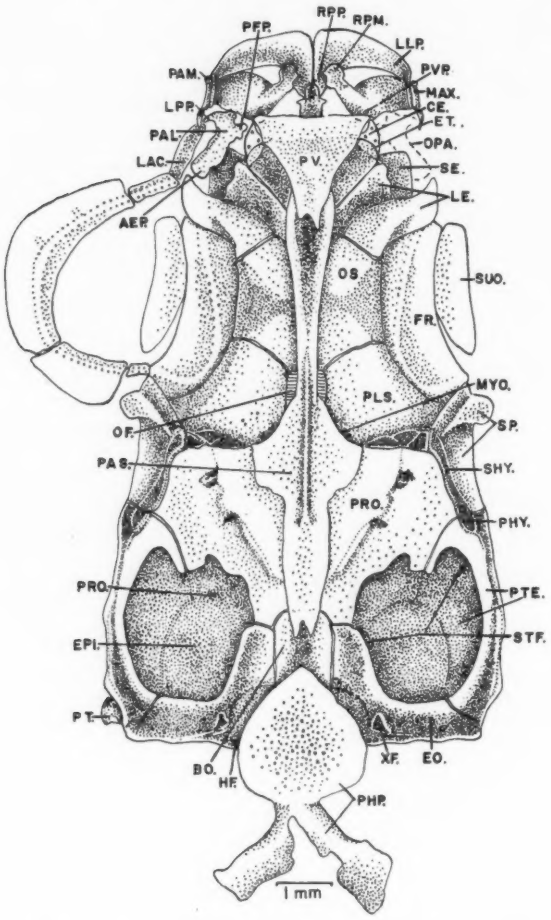


7. Ventral view of the skull of *Rasbora caverii* (Jerd.); suborbitals and palatine shown on one side.



8. Ventral view of the skull of *Esomus barbatus* (Jerd.).

of the auditory region. Dorsally the postorbital and the temporal sensory canals (figs. 1, 2 and 3, tc) are noticed in it and the latter becomes continuous with a triradiate (see, however, below) sensory canal ossicle (supratemporal) which leads mesially into the occipital and posteriorly into the lateral line canals. On the ventral aspect, the bone is noticed to bound the subtemporal fossa (stf) laterally and partly anteriorly and dorsally. In the sectional view of the skull (fig. 11), the bone is noticed as a small projection (pte) between the dorsomedian portion of the sphenotic (sp) and prootic (pro) and accommodates the lateral sensory canal (poe).

Externally, the sphenotic and pterotic jointly show a facet for the articulation of the head of the hyomandibula; the fossa for this in the sphenotic has

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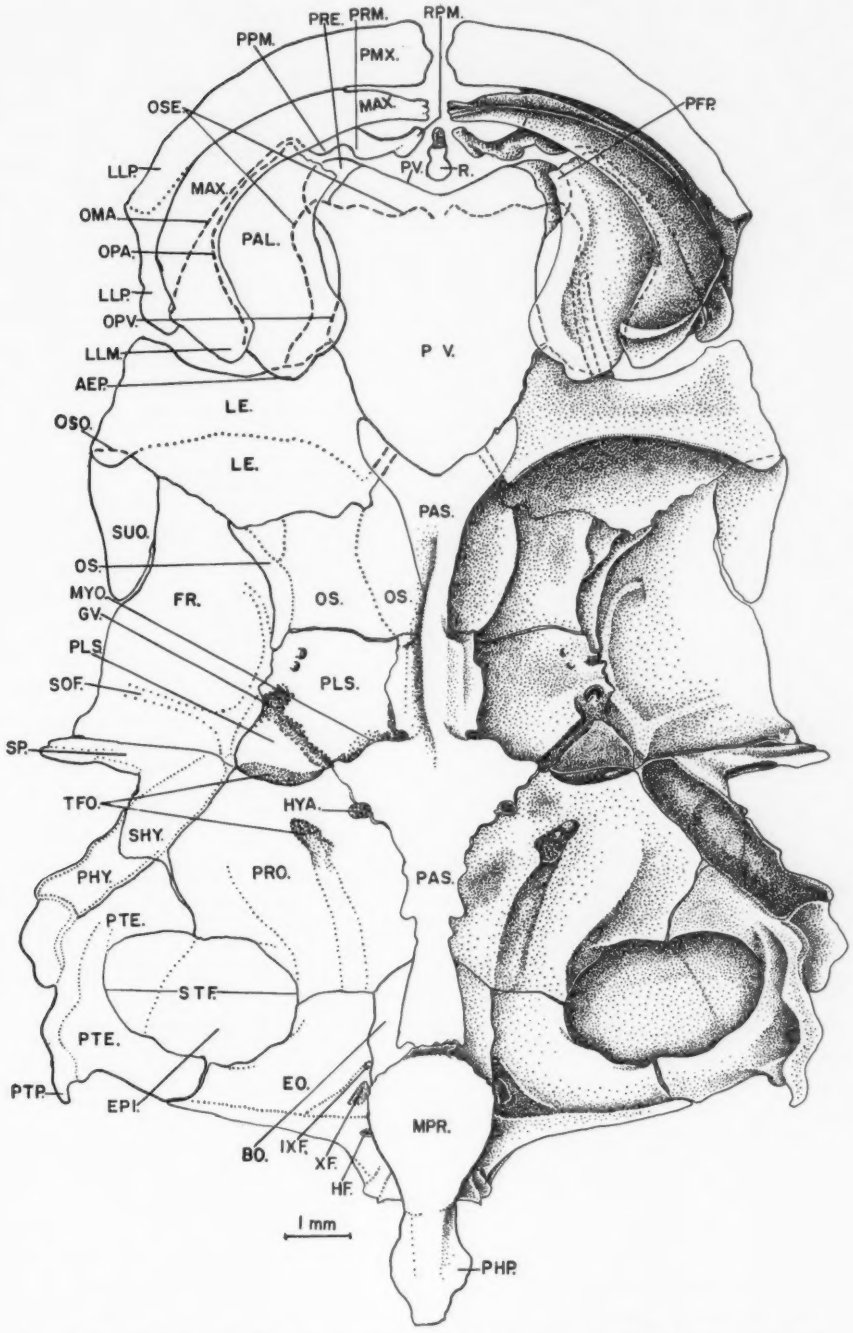
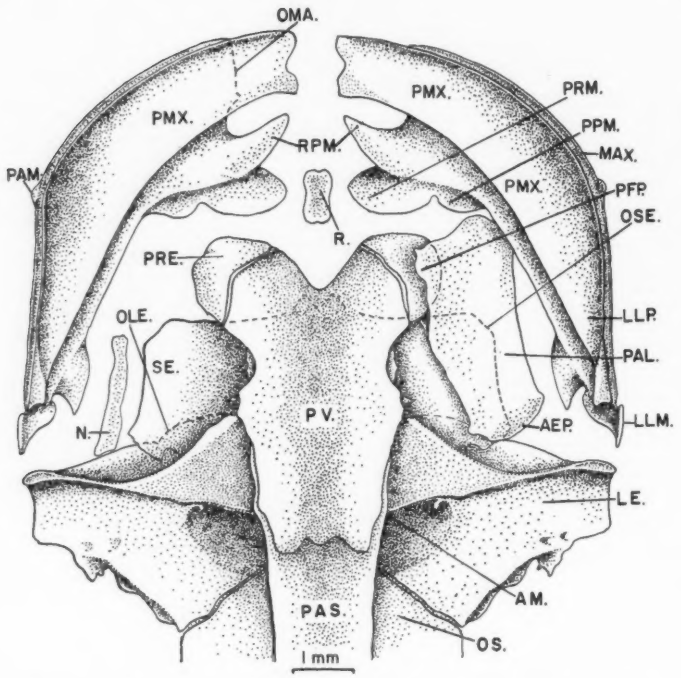


9. Ventral view of the skull of *Garra lamta* (Ham. Buch.); suborbitals not shown.



10. Ventral view of the ethmoid region of *Varicorhinus beso* Rüppel.

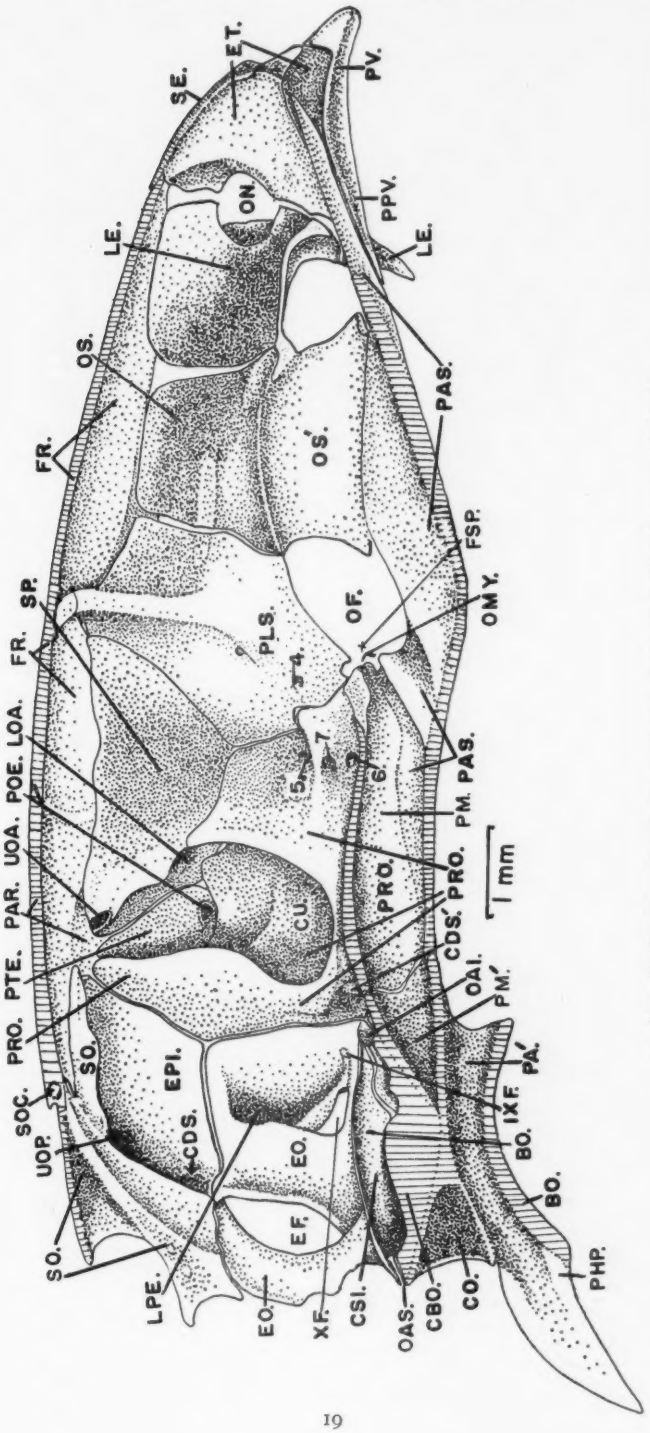
already been referred to; that in the pterotic (phy) is generally short; however, *Rasbora* (fig. 7, phy) shows a comparatively long pterotic fossa.

In a number of examples (*Crossochilus, Cyprinus* etc.) posterolaterally from the pterotic there is a broad or spinous projection (figs. 1, 2, 7 and 9, ptp).

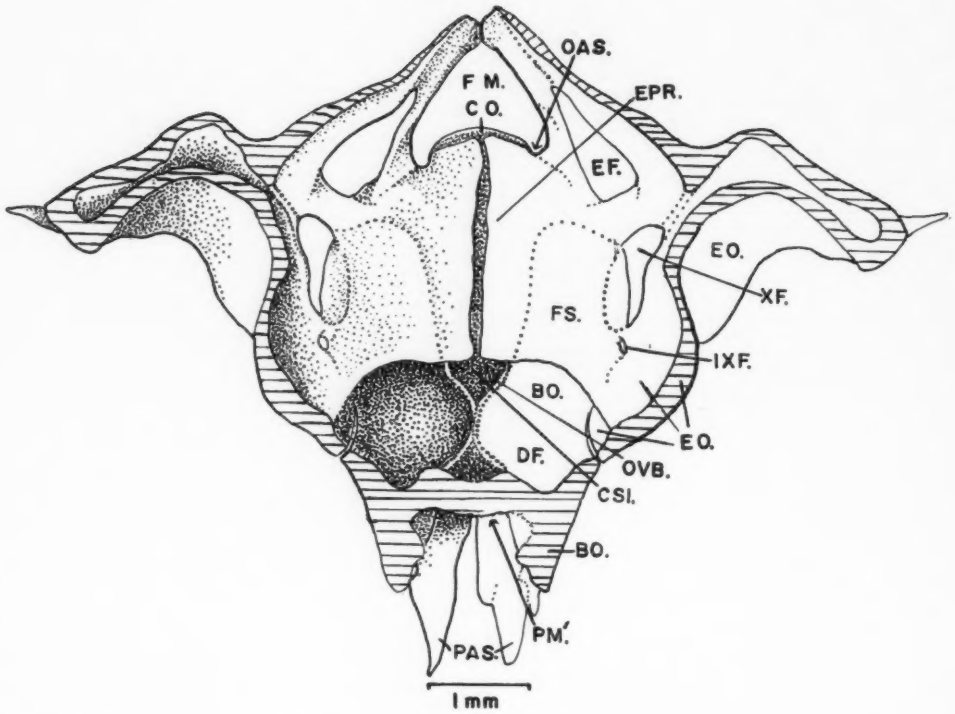
The epiotic (figs. 1, 2 and 4, epi) situated posteriorly between the pterotic and the exoccipital, accommodates the posterior semicircular canal (fig. 11, uop). Generally, a limb of the posttemporal (figs. 1, 2 and 3, pt) comes in contact with the epiotic (epi). In *Cyprinus* there is a posterior projection called the epiotic lamella; in the same example, there may be an internal projection from the epiotic into the cranial cavity. In the ventral view, the epiotic (figs. 4, 5, 7, 8 and 9, epi) is also noticed to form the roof of the subtemporal fossa (stf).

The opisthotic is normally absent; however, in some examples like *Oricnus* and *Schizothorax* there is a well developed irregularly shaped opisthotic ventrally at the meeting of the exoccipital and pterotic bones. Girgis ('52, fig. 3) labelled a portion as opisthotic in the posterior region of the skull of *L. horie*; this probably is a portion of the exoccipital.

The disposition of the semicircular canals and their openings are best seen



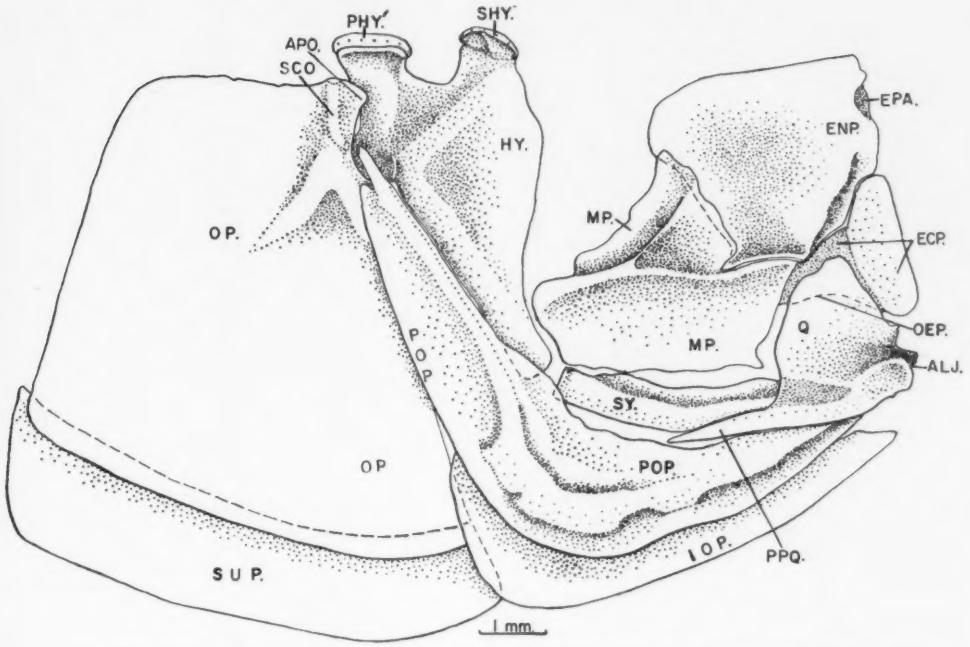
11. Longitudinal sectional view of the skull of *Labeo boggut* (Sykes).



12. Occipital segment of *Scardinius erythrophthalmus* Linn. with the supraoccipital removed.

in the longitudinal section of the skull (fig. 11). The upper (uoa) and lower (loa) openings for the anterior semicircular canal are noticed in the sphenotic (sp); the anterior opening (poe) for the lateral canal is seen in the pterotic (pte); the upper opening (uop) for the posterior semicircular canal is noticed in the epiotic (epi); the posterior opening for the lateral and the lower opening for the posterior (lpe) are jointly seen in the exoccipital (eo), just above the vagus foramen (xf). There is also a blind *cul-de-sac* (cds) in the epiotic below the foramen (uop) for the posterior semicircular canal.

Over the pterotic, there is a canal bone (figs. 1, 2 and 3) establishing connexion between the temporal (tc), occipital (oc) and lateral line sensory canals. This is the supratemporal (st) and shows at least two types of arrangement. The bone may be triradiate; the mesial limb connects the occipital canal, and the lateral limb the temporal canal, while the posterior limb is in contact with the canal bone on the posttemporal (pt). This arrangement is seen in forms like *Labeo, Cirrhina* etc. In some, one of these limbs may be reduced in size (*Rasbora,* fig. 2, st; *Schizothorax, Orienus, Barilius*) so that the triradiate appearance is lost. In *Esomus* (fig. 3, st), *Chela, Leuciscus, Moapa* etc., the



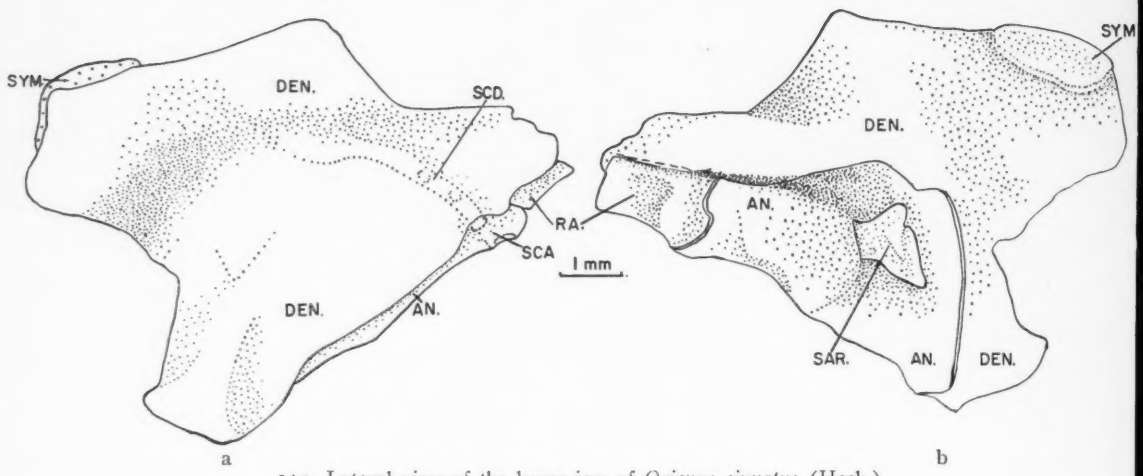
13. The upper jaw and opercular bones of *Rasbora caverii* (Jerd.), palatine omitted.

supratemporal is a cylindrical bone anteriorly in contact with the occipital and temporal canals and posteriorly with the canal in the posttemporal (pt).

In *Cyprinus*, however, GOODRICH ('09, p. 346, fig. 327) delineated two supratemporals, the posterior of which has a limb articulating with the epiotic; he has, however, later called this bone a tabular (?) ('30, p. 299, fig. 305). More correctly, the posterior one should be called a posttemporal as has been done by GREGORY ('33, p. 190, fig. 74).

A reference should be made to the posttemporal. The posttemporal (fig. 2, pt) by its mesial limb comes in contact with the epiotic and by its lateral limb with the supracleithrum. In *Esomus* (fig. 3, pt), however, an anterior projection from the lateral limb of the posttemporal (pt) is also seen.

The supraoccipital (figs. 1, 2 and 3, so) forms the roof in the median region of the cranium behind the parietals; however, the two exoccipitals extend over the foramen magnum posterior to the supraoccipital and form the roof for the foramen magnum in this region. The supraoccipital also shows a median membranous projection (sop) posteriorly. The occipital sensory canal (fig. 11, soc) always runs in front of the supraoccipital in the parietal bones (par) and never through the supraoccipital (so); however, in a few gobionine examples (RAMASWAMI, '55) the sensory canal passes through the supraoccipital bone also.



14 a. Lateral view of the lower jaw of *Orienus sinuatus* (Heck.).
14 b. Mesial view of the lower jaw of *Orienus sinuatus* (Heck.).

The exoccipitals (figs. 11 and 12, eo), as noted above, keep out the supraoccipital from bounding the foramen magnum (fm) dorsally. Each exoccipital shows a large lateral fenestra (ef). The exoccipital also mesially bounds the subtemporal fossa; the lateral limb of the exoccipital coming in contact with the mesial limb of the pterotic is described as the 'paroccipital process' in *Labeo* by SARBAHI ('33). He also pointed out that it was likely that the paroccipital process was formed by the opisthotic, which was absent as a separate bone in the adult skull.

The exoccipital accommodates the posterior semicircular canal and the joint posterior opening (fig. 11, lpe) of the lateral and the lower opening of the posterior semicircular canal are seen in the mesial aspect of the skull. The two exoccipitals (fig. 12, eo) show mesially projections (epr) over the basioccipital (bo) and the approximation of these two over the oblique vertical projections (ovb) from the basioccipital, forms a passage, — the cavum sinus imparis (csi) accommodating the sinus impar and the sinus endolymphaticus. Posteriorly, through an opening (oas) between each exoccipital and the basioccipital (con), the sinus impar gains exit to be enclosed by the first two weberian ossicles. Laterally to this median cavum, there are the right and left fovea sacculi (fs); each chamber is blind posteriorly and opens anteriorly (fig. 11, oai) and is formed by the basi and ex-occipitals. The foveae accommodate the sacculi. In front of the opening of the fovea for the sacculus, there is also a blind *cul-de-sac* (cds') in the prootic bone. In each sacculus a large otolith (sagitta) is seen.

The basioccipital shows ventrally a large bony plate (masticatory process: fig. 5, mpr) for the attachment of a horny pad and posteriorly, a process

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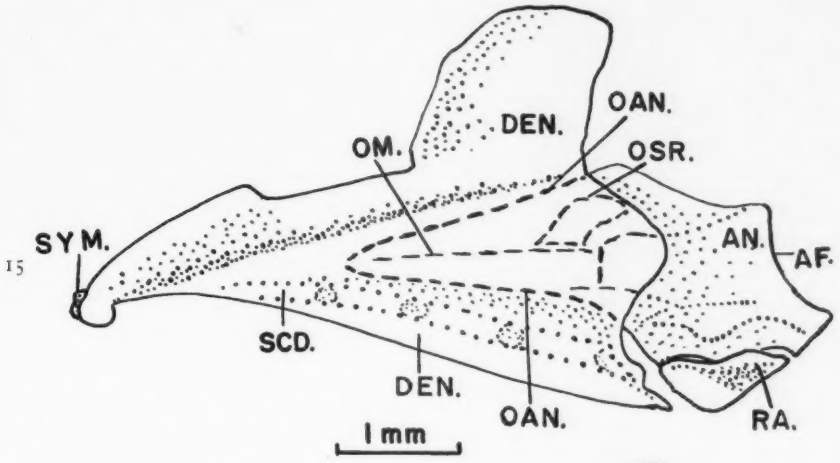

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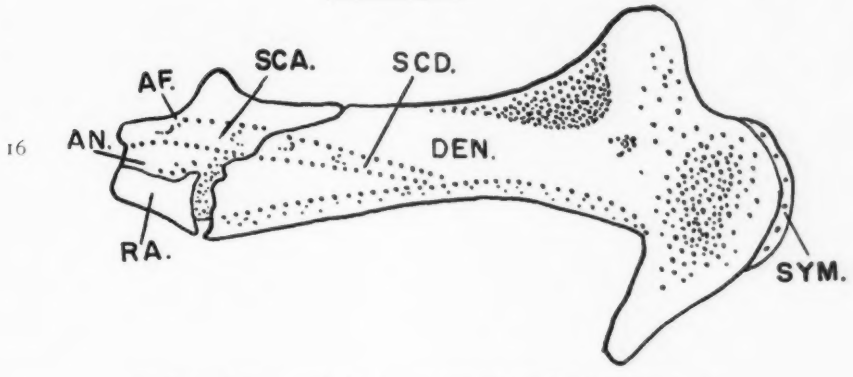

15. Lateral aspect of the lower jaw of *Barbus khudree* Sykes.
16. Lateral aspect of the lower jaw of *Labeo rohita* (Ham. Buch.).

which may be bifid as in *Esomus* (figs. 3 and 8, php); the masticatory and the posterior process are together called the pharyngeal process. The pharyngeal process allows the dorsal aorta to pass between it and the basioccipital (fig. 11, pa'). The pharyngeal process is described as being formed by the haemal arches of the third vertebral segment assimilated into the skull; the centrum of this vertebral segment unites with the basioccipital (NUSBAUM, '08) to form the concave condyle (fig. 11, co).

The temporal fossae and the nerve foramina: On the ventral aspect of the skull, in the auditory region, two depressions, the subtemporal fossae (figs. 4, 5, 7, 8 and 9, stf), are noticed for the insertion of the muscles of the branchial apparatus. These fossae are bound externally by pterotic, prootic and exoccipital. Forming the roof of the fossae are noticed the epiotic and pterotic in all the genera and the prootic, parietal and exoccipital also contribute

severally or in combinations towards forming the roof. In none of these, does the sphenotic enter into the formation of the roof of the fossa as shown by SARBAHI ('33) in *Labeo*.

GIRGIS ('52) labelled the subtemporal cavity as 'otic cavity' in his figure 3. This is not correct. Further, in the same figure, he labels a portion in front of the basioccipital as basisphenoid. A basisphenoid is *not* noticed in the forms examined by me, as the bone is absent in these. A portion of the parasphenoid has been identified as basisphenoid by him.

Between the roof of the skull and the posttemporal, there is a space, into which the muscles pass for insertion on the skull. REGAN ('11) described this space as a posttemporal fossa and considered the possession of a large fossa as a generalised character. In some form or other, all the genera possess a posttemporal fossa in Cyprininae.

The orifices for the exit of the cranial nerves have been described by SARBAHI ('33) in *Labeo* and I am making a brief reference to them here.

1. The foramen for the olfactory nerve (fig. 11, on) is in front of the lateral ethmoid (le) from which it gains exit to innervate the olfactory organ.

2. In front of the posterior myodome, an orifice (of) bounded by the parasphenoid (pas), orbitosphenoid (os') and pleurosphenoid (pls) serves for the exit of the optic nerve; the posterior myodome is lateral to this orifice.

3. I shall consider the third, fourth and sixth pairs of cranial nerves together. The oculomotor passes into the orbit through the pituitary fossa to innervate the inferior oblique, superior, inferior and anterior recti muscles. SARBAHI ('33) described the oculomotor foramen in *Labeo* as 'a small elongated slit-like aperture on the floor of the myodome, situated about quarter of an inch behind the optic foramen on each side'. The fourth or trochlear nerve gains exit through an orifice (fig. 11, 4) in the pleurosphenoid (pls). This orifice is more towards the optic foramen in the bone. In *Rohu*, the pathetic foramen 'is a very narrow elongated foramen towards the lower end of the alisphenoid above the optic foramen and beneath the foramen for the ophthalmic division of the trigeminal nerve' (SARBAHI, '33). The sixth or abducens nerve passes through a small orifice (fig. 11, 6) into the myodome and innervates the posterior rectus. In *Rohu* the abducens foramen is a small one on the floor of the myodome in between the oculomotor and trigeminal foramina.

4. In the mesial face of the anterior portion of the prootic bone, there is a ridge laterally to the myodome, separating an orifice into two; the one to the right gives exit to the ophthalmic branches of the trigeminal and facial nerves. The left opening (fig. 11, 5) is for the principal branches of the trigeminal nerve. The hyomandibular branch of the facial comes out of a foramen (fig. 11, 7) in the prootic, separated from the trigeminal opening by the lateral commissure.

5. The ninth nerve usually has an independent orifice (figs. 4, 5, 7, 8, 10,

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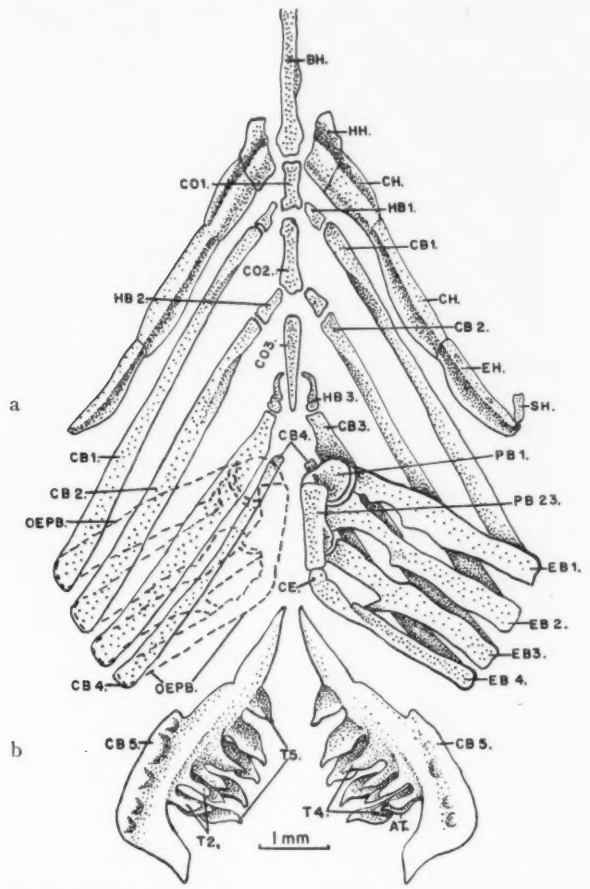

17 a. Hybobranchial apparatus of *Rasbora caverii* (Jerd.).
17 b. The inferior pharyngeal bone of *Rasbora caverii* (Jerd.) showing teeth.

11 and 12, ixf) in front of the vagus foramen (xf); in some forms like *Catla*, these two orifices may be combined and there is a jugular foramen in the posterior region of the exoccipital.

6. The exoccipital also shows a small hypoglossal foramen (figs. 4, 5, 8 and 9, hf) posterior to the vagus foramen.

The upper jaw and opercular bones: In the cyprinids, the upper jaw bones show an uniformity of structure. Of the opercular series, the opercular (fig. 13, op) is vertically elongated and shows an unindented upper edge and towards the hyomandibula, there is a passage (sco) in the bone for the mandibular sensory canal in the majority of cases examined; in *Notropis, Moapa, Hyborhynchus, Eremichthys, Aphyocypris, Aspius, Esomus* and *Diptychus* such a passage is not seen. Similarly the opercular may show a pointed process

towards the hyomandibula (*Garra, Crossochilus, Cirrhina, Labeo* etc.) while in some it may be a blunt process (apo) or even absent. The preopercular is the large archlike bone carrying the sensory canal in it. It has already been stated that the temporal canal and the mandibular sensory canal (running through preopercle) are connected through a passage in the opercular in some examples; in *Aphyocypris* there is no canal in the opercle and the preopercle is so elevated that its tubular upper end comes near the temporal canal. In *Aristichthys* also the preopercle is elevated but the opercle shows a sensory canal in it. The subopercle (fig. 13, sup) and interopercle (iop) do not show any variation except in size.

The hyomandibula gains articulation with the sphenotic-pterotic region by two facets, an anterior (fig. 13, shy') with the sphenotic and a posterior (phy') with the sphenotic-pterotic region. These two may also closely approximate with each other as in *Labeo* so as to show only a single facet. The symplectic is a unique bone (sy) in the upper jaw of the majority of fishes; it is wedgeshaped and is usually long in the cyprinids. In forms like *Orienus, Schizothorax, Cirrhina, Labeo, Diptychus, Varicorhinus* and *Barilius* the bone is considerably short; in *Orienus*, it firmly articulates with the metapterygoid. In *Ptychidio* the symplectic has a prominent alveolar portion articulating by a dentate suture in the mesial face of the metapterygoid and a short projecting limb articulating with the quadrate. Generally, in fish, the quadrate (q) has a backwardly directed process (ppq) laterally to symplectic; in *Orienus* this process, however, is mesially disposed. In *Barilius* the lateral posterior process of quadrate is short and blunt. In *Aspius*, the quadrate bone is comparatively short. The ecto- (ecp), ento- (enp) and meta-pterygoids (mp) form the anterodorsal portion of the upper jaw; the entopterygoid articulates with the palatine by a facet (epa). There may be a large gap between the metapterygoid and quadrate. REGAN ('11) noted the occurrence of a large gap between the quadrate and the metapterygoid in *Opsariichthys* and *Chela*; I have also noticed the gap in these two forms. The characins posses such a gap; also, I have noticed a similar gap in the cobitid *Acathopsis* and *Acanthophthalmus* (RAMASWAMI, '53).

The lower jaw: The shape of the lower jaw is not uniformly the same in the Cyprininae examined by me. There are four bones forming each ramus of the jaw, viz., a dentary (figs. 14 a, 14 b, 15 and 16, den), an angular (articular *autt.*) (an), a sesamoid angular (sar) and a retroarticular (angular *autt.*) (ra) which latter is the only cartilage bone of them. The sensory canal is associated with the dentary (scd) and the angular (sca) and is always laterally disposed in these bones. While describing the lower jaw bones of *Labeo*, SARBAHI ('33) referred to a dentary, an angular (= retroarticular) and an articular (= angular) only; he apparently missed the mesial ossification sitting upon Meckel's cartilage, — the sesamoid angular. Sometimes in the

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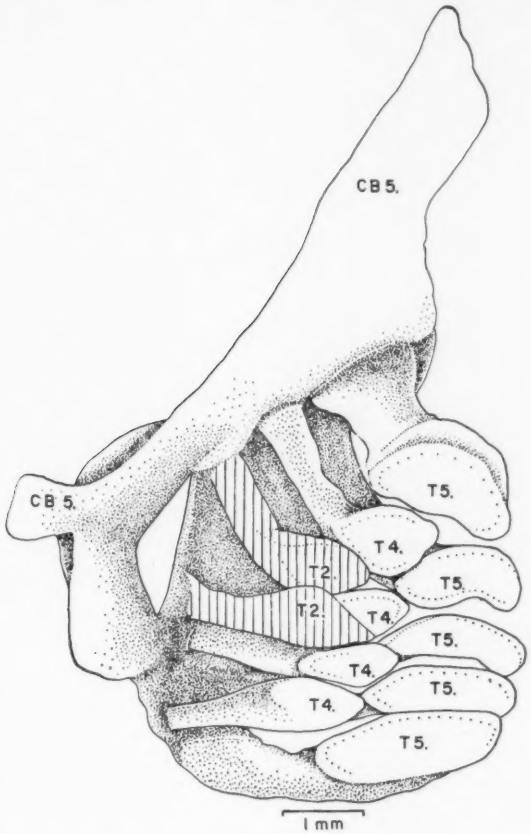
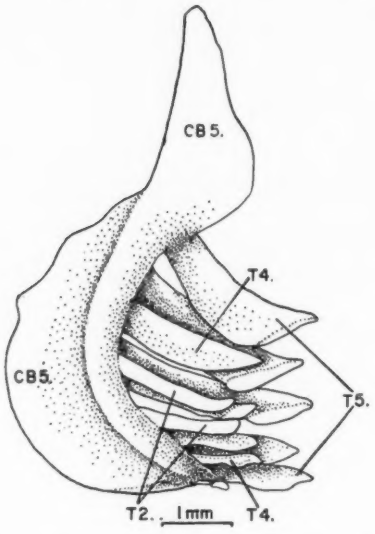


18. The inferior pharyngeal bone of *Labeo diplostomus* (Heck.).

cyprinines studied, a small or large gap may be noticed in the edentulous dentary (*Aphyocypris, Aristichthys*); also while the bone is fairly long in the majority of the genera, the dentary is short in *Garra* and *Orienus*. In some it may also give rise to a process anteroventrally as in *L. rohita* (fig. 16) and *Orienus*. In *Barbus* (fig. 15), *Chela* etc., a prominent coronoid process is seen from the dentary.

The hyobranchial apparatus: An examination of the hyobranchial apparatus of the Cyprinidae reveals a striking uniformity of structure. In the branchial apparatus (figs. 17 a and 17 b) two (*Labeo*) or three (*Orienus, Leuciscus, Hyborhynchus* etc.) median copulae (col, co2, co3), three pairs of hypobranchs (hb1—hb3) and two pairs of pharyngobranchs (pb1, pb23) are present. The first pharyngobranch is small and belongs to the first arch; the larger second is a composite one, probably representing the fused pharyngobranchs of the second and third arches (pb23). The fourth arch is without one. However, in



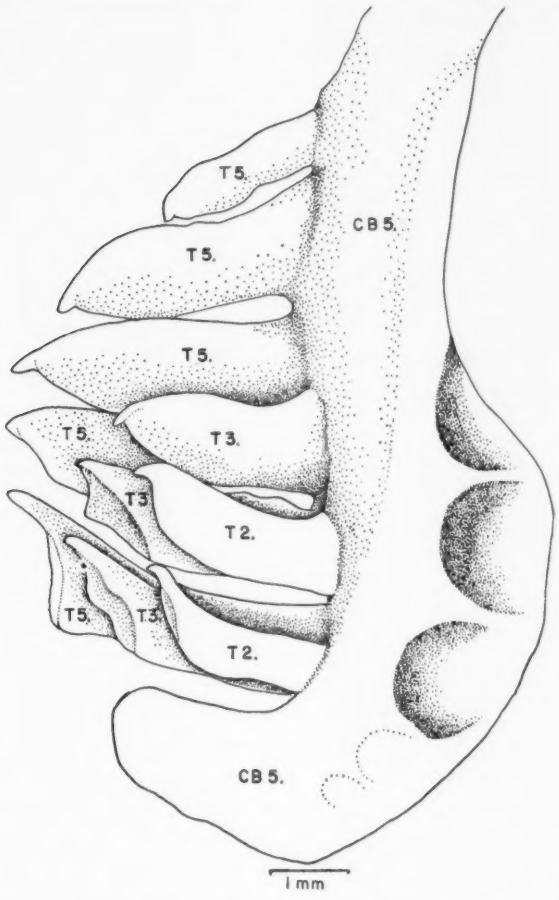
19. The inferior pharyngeal bone of *Tylognathus klatti* Kosswig.

Esomus, a calcification in the epiphysial cartilage of the fourth epibranch occurs. In his figures of *Rohu* branchial arches, SARBAHI ('33, p. 399, fig. 24) draws a cruciform basibranch and posterior to it, a similar *median* bone has been labelled the third hypobranch. My alizarin transparencies of *L. rohita* show that the second cruciform bone (labelled third hypobranch by Sarbahi) is really the second copula (representing the fused basibranchials of the third and fourth arches) and there is a separate pair of hypobranchs belonging to the third branchial arches.

There are only two pharyngobranchs (edentulous) as described above. I have examined five species of *Labeo* including *rohita* and I have failed to discover the fourth pharyngobranch delineated by Sarbahi. Probably the cartilaginous epiphysis of the fourth arch is mistaken by him for the pharyngobranch.

GIRGIS ('52) in describing the hyobranchial apparatus of *L. horie* noted that in the fifth branchial arch a small cartilaginous portion represented the fifth ceratobranch, and the longer bony portion, an epibranch. I have not noticed such an arrangement in the specimens of *horie* and *niloticus* studied by me at the British Museum (N.H.), London. There is only the large bony arch, — the fifth ceratobranchial (also called the inferior pharyngeal bone) with the characteristic pharyngeal dentition.

The pharyngeal dentition and the shape of the teeth in the Cyprinidae are noticed to vary considerably; age plays a prominent part in changing the shape of teeth. VASNECOV ('38) has given an excellent account of the development of pharyngeal teeth in a few cyprinids.



19 a. The inferior pharyngeal bone of juvenile *Varicorhinus beso* Rüppel.

Listed below are a few genera of the ones examined by me with one, two and three rows of pharyngeal teeth:

One row:	*Eremichthys*	4—4 (4—5, according to HUBBS & MILLER. '48)
	Hyborhynchus	5—5
	Moapa	4—4
Two rows:	*Aphyocypris*	4, 4—4, 4
	Leuciscus	5, 2—2, 5
	Notropis	4, 2—2, 4
	Phoxinus	5, 2—2, 5
	Ptychidio	5, 4—4, 5
Three rows:	*Cyprinus*	1, 1, 3—3, 1, 1
	Labeo	5, 4, 2—2, 4, 5 (fig. 18)
	Orienus	4, 3, 2—2, 3, 4

Rasbora	5, 4, 2—2, 4, 5[1] (fig. 17 b)
Tylognathus	5, 4, 2—2, 4, 5 (fig. 19)
Varicorhinus	5, 3, 2—2, 3, 5 (fig. 19 a)

Generally the Asiatic Cyprininae are provided with three rows of teeth, those of the outermost row being the biggest; they may be spoonshaped or flattened due to usage. Among the gudgeons examined by me (RAMASWAMI, '55), there is one form, *Hemibarbus,* which showed *three* rows of teeth while the rest possessed one or two rows; in this feature, *Hemibarbus* is far removed from the other gudgeons. Not a single Indian or African cyprinine genus is known to have only a single row of teeth, as far as I am aware.

Weber's apparatus: In the Cyprininae, the gasbladder is not enclosed in a bony capsule and is coupled with the inner ear by a chain of ossicles. CHRANILOV ('27) has given a comprehensive account of the weberian apparatus in the Cypriniformes. SARBAHI ('33) described the apparatus in *L. rohita.* WATSON ('39) described the development of the first four vertebrae and ossicles in the goldfish. MOOKERJEE, GANGULI and MUKERJI ('52) have described the adult weberian apparatus of *Esomus.*

According to SARBAHI ('33), the opisthocoelous first vertebra in *Rohu* carries a pair of transverse processes and the neural arch is represented by the claustra and scaphia capped by a 'keystone' arch with a small neural spine. The second and third centra fuse to form a biconcave large centrum; anteriorly a pair of transverse processes (of the second vertebra) arise and the tripus articulating with the third centrum, probably represents its transverse process. The neural arches of the second and third vertebrae are fused exhibiting, however, three different portions. The amphicoelous fourth vertebra shows the neural arch and spine and from the centrum there arise the stout lateral and ventromesial processes. These processes representing the transverse processes of the fourth vertebra form a vertical wall in front of the gasbladder.

Unfortunately Sarbahi had no access to CHRANILOV's paper ('27) and therefore, the discrepancy in the nomenclature adopted by him.

CHRANILOV ('27) figures the weberian apparatus of *Cyprinus* which has been reproduced by BERG ('40) with certain modifications in the nomenclature and I shall now describe the apparatus according to Berg. The first centrum shows a pair of short parapophysis (= transverse processes of Chranilov); the claustrum represents the neural spine and the scaphium the neural arch of this vertebra. The incus (= intercalarium) represents the neural arch of the second vertebra and there is a large neural spine. The malleus (= tripus) represents the parapophysis of the third vertebra and the neural arch and spine are distinct, the latter being very large. The fourth vertebra shows a comparatively smaller neural arch and spine; the parapophyses are elongated

[1] In the figure of the inferior pharyngeal bone of *Rasbora* drawn, on one side is shown an extra tooth, probably belonging to the second row.

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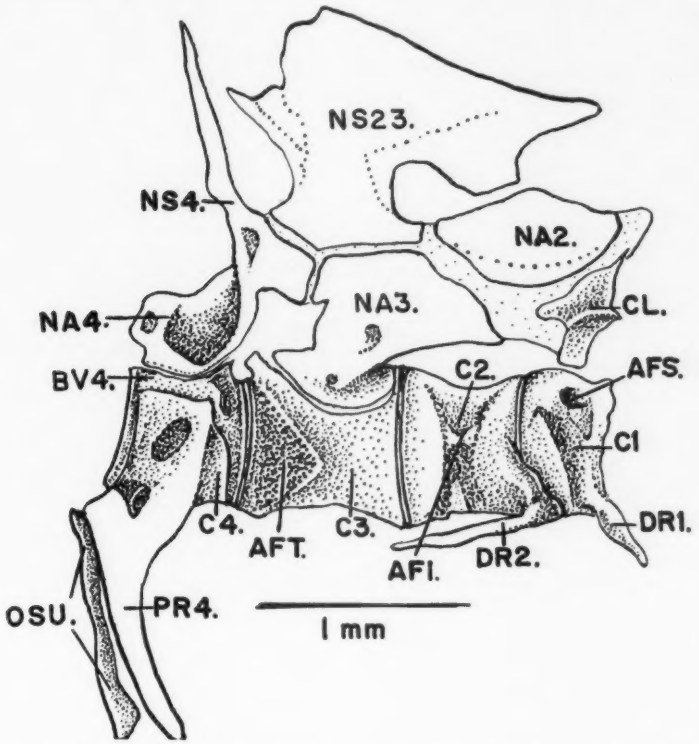
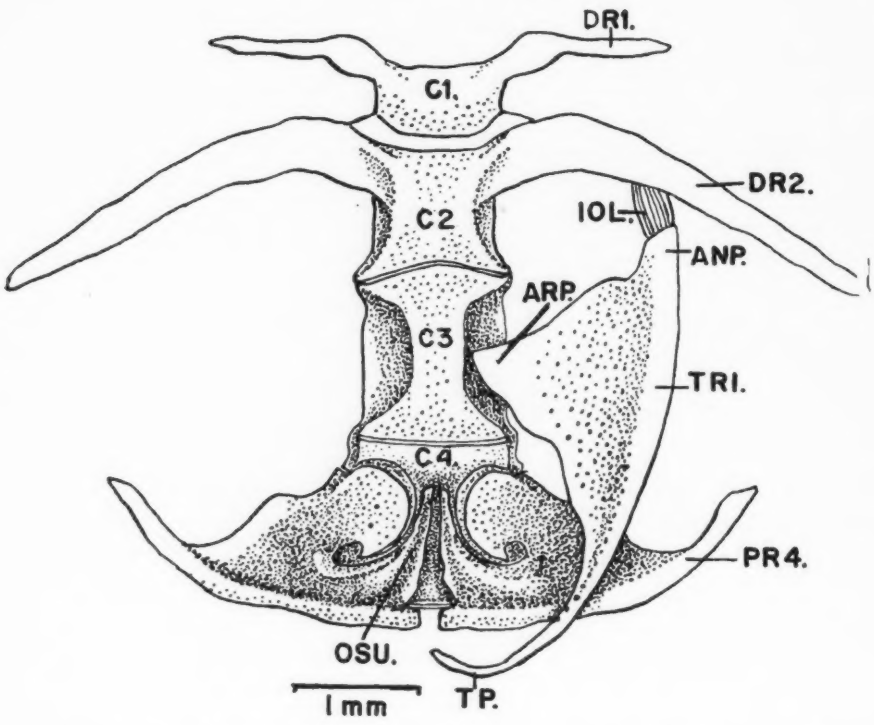


20. Lateral view of the first four vertebrae of *Notropis cornutus*; of the ossicles, only claustrum is shown.

and directed ventrally and from the mesial aspect of each parapophysis, there arises a process which comes in contact with a similar process from the opposite side. These processes representing ribs, are the ossa suspensoria.

I have already described the weberian apparatus of *Garra* (RAMASWAMI, '52) following the nomenclature employed by WATSON ('39) and NELSON ('48). However, I am now describing the apparatus in *Notropis*, an American species. The first centrum (figs. 20 and 21, cl) shows an elongated pair of dorsal ribs (drl) (transverse process [CHRANILOV, '27]; parapophysis [BERG, '40]; lateral process [MOOKERJEE, GANGULI, MUKERJI, '52]). This has no neural arch. Both Chranilov and Berg labelled the neural arch occurring above the claustra and scaphia as that of the second vertebra, thereby noting the absence of the first arch. Following WATSON ('39), it may be inferred that the basidorsal of the second vertebra not only forms the second neural arch (na2) but also the intercalarium. However, in *Esomus*, MOOKERJEE, GANGULI, MUKERJI ('52) have shown united neural arches of the first three vertebrae. The claustrum described by Berg as the neural spine of the first vertebra is an independent



21. Ventral view of the first four vertebrae of *Notropis cornutus* with tripus on one side.

membranous ossification in that region according to Watson; the ascending and articulating processes of the scaphium are formed by the basidorsal of the first vertebra and the concha stapedis by a separate mesenchyme disc. Thus SARBAHI ('33) is incorrect in designating the 'keystone' arch above the claustrum and scaphium as the first neural arch; it ought to be labelled the second neural arch. The second and third centra may be fused. While in the majority of cyprinids examined, the articulation between the second and third centra is not visible, in *Esomus*, *Notropis* (fig. 21), *Ptychochilus*, *Opsariichthys*, *Aphyocypris* and *Aspius*, it can be clearly made out. However, in *Esomus*, the 'centra of the first four vertebrae are fused' according to MOOKERJEE, GANGULI, MUKERJI ('52). The formation of the neural arches in the second and third vertebral region is very interesting. According to WATSON ('39), above the 2—3 vertebrae, during development, lies a large cartilaginous mass. When ossification sets in, it forms the neural arches and spine of the 'compound vertebra'. The compound cartilage represents according to him, the fused neural spines and the basidorsals of the second, third and fourth vertebrae together with the first three interspinous bones and possibly

the neural spine of the first vertebra also. The participation of the second basidorsal into this was only inferred and not seen as the neural arch of the second vertebra arose out of this mass. Obviously a part of the second basidorsal also gives rise to the mesial part of the intercalarium. When ossification sets in, clear sutural demarcations arise between the neural arches etc., of the second, third and fourth vertebrae.

It should be observed that the neural arch (fig. 20, na2) of the second vertebra is not in line with those of the third (na3) and fourth (na4) vertebrae in the adult; it is situated in line with a clearly demarcated upper portion of the third vertebra (ns23). This upper portion represents according to SARBAHI ('33) part of the neural arches and spines of second and third vertebrae; the lower portion is also apparently formed by the same neural arches. Both CHRANILOV ('27) and BERG ('40) have, however, labelled that upper portion of the third arch in line with the second neural arch as '3rd neural arch'. GOODRICH ('30, fig. 599), however, while not labelling the socalled '3rd neural arch', considered the lower portion as probably representing the second basiventral. It is very likely that the portion of the third vertebra in line with the fourth neural arch is the neural arch of the third vertebra (fig. 20, na3) and the portion above it, the conjoint neural spines of the second and third vertebrae (ns23).

From the centrum of the second vertebra, there projects laterally a pair of large dorsal ribs (figs. 20 and 21, dr2). The third vertebra is devoid of ribs and its basiventral (= pleural rib according to WATSON ['39]) is modified into tripus (fig. 21, tri).

Behind the third vertebra is the fourth (fig. 20) whose neural arch (na4) and spine (ns4) are in line with those of the third and from its centrum project laterally a pair of ribs (pr4) (transverse process [CHRANILOV, '27]; lateral process [SARBAHI, '33]; MOOKERJEE *et al*, '52; parapophysis [BERG, '40]). GOODRICH ('30) regarded them as pleural ribs and it is likely they represent those. From the same centrum, ventrally, there project a pair of processes, — the ossa suspensoria (figs. 20 and 21, os) coming together mesially and forming an osseous boundary for the anterior part of the gasbladder. The transformator process (tp) of each tripus is in contact with the gasbladder posterior to the os suspensorium. The ossa represent the pleural ribs of the fourth vertebra according to Chranilov and Berg. WATSON ('39) however, has shown in the goldfish that from the fourth pair of basiventrals, there project two pairs of processes, the ossa suspensoria and the pleural ribs. Obviously according to him, the ossa suspensoria are different from the pleural ribs and has derived them from the haemapophyses of that segment.

DISCUSSION

It was noted that the family Cyprinidae was divided into a number of subfamilies; some of the genera examined by me can be allocated under those subfamilies as follows:

Cyprinina: *Barbus, Capoeta, Carassius, Catla, Cirrhina, Crossochilus, Cyprinus, Diptychus, Garra, Hyborhynchus, Labeo, Orienus, Thynnichthys, Tylognathus.*
Rasborina: *Rasbora, Amblypharyngodon, Aphyocypris, Esomus,*
Xenocypridina: *Mystacoleucus.*
Daninina: *Danio, Barilius, Opsariichthys.*
Leuciscina: *Leuciscus.*
Abramadina: *Chela.*

Berg ('40) included all these genera under one subfamily Cyprinini. We may now assess if the skeletal characters lend support to the splitting of the members as outlined above.

The premaxillae show two types of arrangement. In one type, including the foreign genera examined, the rostral processes of the premaxillae are directed towards the rostral in the majority of forms; in *Diptychus* and *Ptychidio*, the rostral process is absent.

The preethmoid bone shows variation also. In forms like *Amblypharyngodon, Aristichthys, Esomus, Leuciscus, Phoxinus, Notropis* and *Scardinius,* no ossification is noticed in the cartilaginous epiphysis extending from the anterior tip of the prevomer to the ethmoid; a preethmoid bone is therefore, absent in these forms. In *Labeo* and the majority of forms, a small ossification is noticed laterally in the cartilaginous epiphysis and this has been identified as a preethmoid. In forms like *Ptychidio* and *Varicorhinus,* the entire epiphysis appears to undergo ossification and is labelled preethmoid.

The maxilla holds the premaxilla in a fork as it were and the two processes of the fork are the dorsal and rostral processes. On the posterior face of the mesial limb of the maxilla, there are usually two articular facets; the more mesial comes in contact with the preethmoid (when present) and the lateral with the palatine. When the preethmoid is absent, the mesial process comes in contact with the prevomer.

In the Gobioninae (Ramaswami, '55) it has been noticed that between the preethmoid and the mesial process of the maxilla, there is usually a fibrocartilaginous disc; this may assume large proportions as in *Saurogobio, Abbottina* and *Pseudogobio* (op. cit.). In *Cyprinus* and *Amblyrhinchichthys* this cartilage undergoes ossification.

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In the genera examined by SAGEMEHL (1891), this cartilage or bone has been called a submaxillary. I find after examining a large number of skulls, little justification for describing it as 'submaxillary' as the term would mean that the bone is disposed ventrally to the maxilla which it is not. It is therefore necessary to call this bone a 'preethmoid' (as has been done by me in describing the Homalopterid Skull [RAMASWAMI, '48]) as the bone is not only in front of the ethmoid but also is a cartilage bone. In a large number of forms where both the 'septomaxilla' and 'submaxillaries' occur, the nomenclature will have to be suitably altered. It would be necessary to call the septomaxillary apophysis of the prevomer the first preethmoid, and the submaxillary in front of this articulating with the maxilla, the second preethmoid as has been done by me and the second submaxillary as the prepalatine. If the preethmoid of *Labeo* (GREGORY, '33) or of *Pogonichthys* (STARKS, '26) extended anteriorly and became independent, it would represent the 'submaxillary' of Starks. The ambiguous term 'submaxillary' may be dropped. In this connection, the observation of STARKS ('26, p. 332) is very significant:

> In the latter [Eventognathi] it [preethmoid] seems to arise from the submaxillary cartilage that connects it with the maxillary. The preethmoid and the submaxillary show the same varied degree of ossification. If these two are not ossifications of the same cartilage the influence of ossification seems to extend from one to the other. In *Catostomus* the submaxillary may be entirely cartilage; it may have ossified at both ends; or it may be ossified for a considerable extent throughout its length. In *Misgurnus*, it is completely ossified. The preethmoid in the Eventognathi perhaps arose as epiphysis to these bones and then formed seats of articulation.

In the orbitotemporal region, the lateral ethmoid and supraorbital may be considered together. The lateral limb of the lateral ethmoid may show projections anteriorly and posteriorly, though small as in *Esomus*, *Garra* etc., while in *Labeo*, only the anterior process is seen. In the majority of forms the processes are absent. The supraorbital may be large extending as far as the last suborbital ossicle as in *Rasbora* and *Aphyocypris* or they may be short as in the majority of Cyprininae examined.

In discussing the primitive characters of *Opsariichthys*, REGAN ('11) referred to the possession of a 'complete series of circumorbitals' as a generalized character. I have not noticed even a single instance of incompleteness of the circumorbitals in the genera examined by me. I am unable to follow what Regan meant by 'complete series'.

The orbitosphenoids are usually double; the two orbitosphenoids may mesially come together and may give rise to a process which comes in contact with a similar process from the parasphenoid to form the interorbital septum (*Labeo*,

Mystacoleucus etc.). In examples like *Cyprinus* and *Carassius* the orbitosphenoids have come together and fused mesially so that the bone is single and in these, no median process is given off towards the parasphenoid to form the interorbital septum. In *Scardinius* the two orbitosphenoids show a fusion posteriorly while separate anteriorly. I have not noticed such fusion in the other genera studied by me. In *Salmo* (DE BEER, '37) the two orbitosphenoids are paired to start with and later on they fuse.

The auditory region of *Esomus* shows a unique feature. The sphenotic is noticed as a roofing bone between the frontal and pterotic while in all the other genera, the frontal meets the pterotic and the sphenotic is ventral and only the sphenotic process is noticed dorsally. Moreover, the temporal canal does not run in the sphenotic in any cyprinid, except however, in *Esomus*, where due to the intercalation of the sphenotic between the frontal and pterotic, the canal runs through it.

The sphenotic and pterotic jointly show a facet for the two (or single, *Labeo*) heads of the hyomandibula.

The subtemporal fossa is a characteristic feature of the cyprinid skull. The fossa is bounded by the pterotic, exoccipital and prootic and roofed partly by the above bones and by the parietal and epiotic; the parietal is noticed to roof the fossa in *Cirrhina* over a very small area. This fossa has been erroneously called 'otic cavity' by GIRGIS ('52) in *Labeo*.

On the dorsal aspect of the skull, between the pterotic and the posttemporal, there is a gap into which muscles are inserted; this cavity is called the posttemporal fossa by REGAN ('11). The possession of this fossa is considered by him a generalized character. I have noticed the fossa in all the genera studied by me.

The exoccipitals and basioccipital jointly give rise to three chambers in the floor, one mesial and two lateral; the mesial chamber is for the sinus endolymphaticus and the sinus impar and the latter proceeds posteriorly to gain exit by two lateral orifices enclosed between the basioccipital and exoccipitals. The lateral chambers are blind posteriorly and accommodate the sacculi of the membranous labyrinth. Each sacculus contains the largest otolith, the sagitta.

The posterior myodome is a spacious chamber bounded by the prootic, parasphenoid and posteriorly by the basioccipital; the chamber ends blindly in the latter bone. The sphenotic and orbitosphenoids do not enter into its formation as recorded by SARBAHI ('33). The anterior and posterior recti muscles enter into the posterior myodome. In the anterior region between the parasphenoid and lateral ethmoid, there is a gap through which the oblique muscles enter and get inserted on the ventral aspect of the ethmoid; this is the anterior myodome.

In the upper jaw, the palatine establishes articulation with the ethmoid region

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of the cranium and thus suspends the upper jaw. In those forms where a preethmoid is not developed, the palatine has a large socketlike articular facet to articulate with the cartilaginous epiphysis; a ligament attaches the palatine facet with the supraethmoid. The palatine may also articulate with the facet on the posterior face of the maxilla and laterally with the lacrimal. Posteriorly it always articulates with the entopterygoid.

The symplectic is long in some forms; in others like *Varicorhinus*, it is short. The gape of the mouth appears to be affected by the size of the symplectic.

The lower jaw shows considerable variation in size and shape. The characteristic four bones are present, viz., the dentary, angular (= articular), retroarticular (= angular) and sesamoid angular. The nomenclature employed is according to HAINES ('38) who has shown that during development the articular has a membranous origin and therefore represents the angular. The dentary may show a large coronoid process more towards the quadrate articulation or a ventrally directed process towards the symphysis. The mandibular sensory canal passes both in the angular and the dentary and probably these two represent compound ossifications.

A uniformity of structure is noticed in the hyobranchial apparatus. There are only two pharyngobranchs; the first one represents probably the pharyngobranch of the first branchial arch and the second similarly, the combined pharyngobranchs of the second and third arches. The fourth arch appears to have no pharyngobranch ossification.

The fifth branchial arch is represented by only the fifth ceratobranch. GIRGIS ('52) described the inferior pharyngeal bone as an epibranch, the ceratobranch being represented by a cartilage in *L. horie*. In the several species of *Labeo* (including *horie* and *niloticus*) examined by me, such an arrangement is not seen.

The pharyngeal dentition varies considerably. While the rows may be reduced from three to two, not a single Indian or African cyprinine examined showed a single row. Some Gobioninae show a single row of teeth. While the teeth are more pointed during youth, as age advances, some of them wear out and show flattened cusps. According to CHU ('35) and VASNECOV ('39) genera with many teeth are more primitive than those with a single row and if this is correct, the entire family Catostomidae with a row of teeth in the lower pharyngeal bone are more recent forms. EATON ('35) has also come to the same conclusion from the view point of jaw mechanism. On the other hand, BERG ('40) and NICHOLS ('43) consider the Catostomidae as more primitive and according to the latter, when these migrated from Yunan to N. America (through the Behring sea landbridge) the gudgeons which are near relatives of the cyprinids, superseded them there. I have shown elsewhere (RAMASWAMI,

'55) that gudgeons like *Saurogobio, Pseudogobio* and *Abbottina* show a few catostomid skeletal characters.

The weberian apparatus does not show much variation. In some genera the second and third centra are not fused; this is considered to be a primitive character by REGAN ('11). The tripus always shows a transformator process. The fourth pair of pleural ribs are broadened out in some cases. In the Catostomidae, the pleural ribs of the second and fourth vertebrae make an intimate dentate suture, while in *Gyrinocheilus* (RAMASWAMI, '52), they are fused.

The general characters of the Cyprinine skull may now be enumerated:

1. The supraethmoid is broad with no median process towards the rostral.
2. The premaxillae keep the maxillae away from the gape of the mouth.
3. Generally, there are no fontanels in the roof.
4. Generally, the frontals and pterotics meet not allowing the sphenotics to be seen dorsally as a roofing bone.
5. The occipital sensory canal always runs through the parietals.
6. The subtemporal fossa is deep.
7. Ventrally, the anterior margin of the prevomer coincides with that of the ethmoid or may extend anteriorly.
8. The inferior pharyngeal bone is the fifth ceratobranchial and carries teeth.
9. The lower jaw always shows a sesamoid articular bone.
10. In the weberian apparatus, the second and fourth pleural ribs do not unite and the tripus shows a transformator process.

If the above characters are compared with those exhibited by a generalized catostomid like *Catostomus*, it is noticed that *Catostomus* differs from the Cyprininae in the following features:

1. the possession of an elongated second preethmoid,
2. the possession of shallow subtemporal fossae,
3. the possession of independent sensory canal bones from the underlying roofing bones,
4. the possession of a single row of pharyngeal teeth,
5. the interlocking of the pleural ribs of the second and fourth vertebrae.

In view of the above differences, it is not likely that the Catostomidae could have given rise to the Cyprinidae.

Moapa and *Eremichthys*, both from the warm springs of Nevada were described as relict cyprinid genera by HUBBS and MILLER ('48). I have examined the skull and weberian apparatus of these two genera with a view to discover any primitive features. The bones of the skull and the structure of

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the weberian apparatus look very much alike those of any other American minnow *Hyborhynchus*, *Notropis* or *Ptychocheilus*. REGAN ('11) considered *Opsariichthys* primitive as it exhibited certain generalized skull characters: triserial pharyngeal teeth, complete series of circumorbitals, large posttemporal fossae and separate second and third vertebrae. *Moapa* and *Eremichthys* do share the above criteria enumerated except the first as they possess uniserial row of pharyngeal teeth. In fact a number of American minnows also exhibit most of these characters and therefore, from the view point of skeleton, *Moapa* and *Eremichthys* do not show any strikingly primitive organization.

After making a study of the skull characters, I have not been able to find a set of them as distinguishing each of the subfamilies of the Cyprinidae. For example, the subfamily Cyprinina comprises examples like *Cyprinus*, *Labeo*, *Garra* etc. In *Cyprinus*, the second preethmoid is ossified, while in the others it is not. In *Garra*, the parasphenoid broadens out at the region of the prevomer but not in *Labeo*. There is such a divergence amongst the genera that one is but forced to confirm the observation of REGAN ('11) that the characters exhibited by the cyprinid genera are not sufficiently diagnostic to group them into subfamilies.

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SUMMARY:

1. The skull and weberian apparatus have been studied in 39 Indian, American and European cyprinine genera with a view to assess their usefulness in systematics.
2. A preethmoid is wanting in *Esomus*, *Leuciscus*, *Phoxinus*, *Scardinius*, *Aristichthys* and *Notropis*. A second preethmoid is a thin strut of cartilage; in *Cyprinus*, it is ossified.
3. The premaxillae exclude the maxillae from the gape of the mouth; the rostral limb of the premaxillae is absent in *Diptychus* and *Ptychidio*.
4. The lateral ethmoid may be devoid of lateral processes.
5. The rostral is present in front of the ethmoid as a rod-shaped bone; it is attached by ligaments with the premaxillae, ethmoid and maxillae. In *Danio*, the rostral is horizontally expanded.
6. The supraorbital is always present showing size variation; in *Labeo* and *Garra*, it is short and in *Ptychidio*, it is short and broad.
7. The orbitosphenoids are paired except in *Cyprinus* and *Carassius* where they are united. A median projection from the two orbitosphenoids may meet a similar projection from the parasphenoid to form an interorbital septum.
8. The posterior myodome is a spacious chamber in the basioccipital; anteriorly the chamber is bounded by the prootic and the parasphenoid.

9. The sphenotic does not form a part of the cranial roof, as the frontal meets the pterotic posteriorly; in *Esomus*, however, the sphenotic forms the roof and the sensory canal passes in it.
10. The supratemporal may be a triradiate or a simple tubular bone.
11. The supraoccipital does not roof the foramen magnum, the two exoccipitals send wings above the foramen to form the roof.
12. The subtemporal fossae are deep; the posttemporal fossae are poorly developed.
13. An opisthotic is noticed ventrally at the region the exoccipital and pterotic meet in *Oreinus* and *Schizothorax*.
14. The quadrate shows a posteriorly directed membranous extension which is laterally disposed; in *Orienus*, it is disposed medially to the adjacent bones. In *Barilius* the posterior process is very short.
15. The inferior pharyngeal bone (ceratobranchial 5) shows one row (*Moapa, Eremichthys*), two rows (*Notropis, Leuciscus*) or three rows (*Oreinus, Cyprinus*) of teeth.
16. In the weberian apparatus, the tripus always shows a transformator process. The centra of the second and third vertebrae may sometimes be separate. The pleural ribs of the second and fourth vertebrae are always separate.
17. Since the cyprinine skull differs from that of *Catostomus* (Catostomidae) in many important characters, it is likely that the Catostomidae may not have given rise to the Cypininae (Cyprinidae).
18. Since *Moapa* and *Eremichthys*, considered to be relict cyprinine genera, exhibit cranial characters similar to many other American minnows examined, they may not be ancient forms.

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LIST OF ABBREVIATIONS:

acp articular facet for the entopterygoid;
af articular facet for the quadrate;
afi articular facet for the intercalarium;
afs articular facet for the scaphium;
aft articular facet for the tripus;
alj articular facet for the lower jaw;
am anterior myodome;
an angular;
anp anterior process of tripus;
apo anterior process of opercular;

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arp articular process of tripus
bh basihyal (glossohyal);
bo basioccipital;
bv4 basiventral of the fourth vertebra.
c1—c4 first four vertebral centrae;
cbo bisected part of basioccipital;
cb1—cb5 ceratobranchials 1—5;
cds cul-de-sac in the supraoccipital;
cds' cul-de-sac in prootic;
ce cartilaginous epiphysis;
ch ceratohyal;
cl claustrum;
co condyle;
co1—co3 copulae 1—3;
csi cavum sinus impar;
cu cavum utriculus.
den dentary;
df depression in basioccipital leading into fovea saccularis;
dpm dorsal process of maxilla;
dr1 dorsal rib 1;
dr2 dorsal rib 2.
eb1—eb4 epibranchials 1—4;
ecp ectopterygoid;
ef fenestra in the exoccipital;
eh epihyal;
enp entopterygoid;
eo exoccipital;
epa articular facet for palatine;
epi epiotic;
epr exoccipital process over cavum sinus impar;
et ethmoid;
eus utricular bulge.
fm foramen magnum;
fr frontal;
fs fovea saccularis bulge;
fsp fossa for pituitary gland.
gv groove for blood vessel.
hb1—hb3 hypobranchs 1—3;
hf hypoglossal foramen;
hh hypohyal;
hy hyomandibula;
hya foramen for hypophysial artery;
hyf foramen for hyomandibular nerve.
iol interossicular ligament;
iop interopercular.
lac lacrimal;
lc lateral commissure;
le lateral ethmoid;
llm lateral limb of maxilla;
llp lateral limb of premaxilla;
loa lower opening of the anterior semicircular canal;
lp process of the lateral ethmoid;
lpe lower opening of the posterior and posterior opening of the lateral semicircular canals;
lpp lateral process of palatine.
max maxilla;
mp metapterygoid;
mpr masticatory process;
myo posterior myodome.
n nasal;
na2—na4 neural arches of second, third and fourth vertebrae;
ns4 neural spine of fourth vertebra;
ns23 united neural spines of second and third vertebrae.
oai opening of lateral fovea sacculi;
oan outline of angular;
oas opening of atrium sinus impar;
oc occipital canal;
oe outline of ethmoid;
oep outline of ectopterygoid;
oebp outlines of epi- and pharyngo-branchs;
of optic foramen;
ole outline of lateral ethmoid;
om outline of Meckel's cartilage;
oma outline of maxilla;
omy entry into myodome;
on foramen for olfactory nerve;
op opercular;
opa outline of palatine;
ope outline of preethmoid;
opm outline of premaxilla;
ops outline of parasphenoid;
opv outline of prevomer;
or outline of rostral;
os orbitosphenoid;
os' orbitosphenoidal portion of interorbital septum;
ose outline of supraethmoid;
oso outline of supraorbital;
osr outline of supraangular;
osu os suspensorium;
ovb oblique vertical projection of basioccipital.
pa passage for the artery;

pa' passage for artery in pharyngeal process;
pal palatine;
pam process for adductor muscle ligament;
par parietal;
pas parasphenoid;
pbl first pharyngobranch;
pb23 united pharyngobranchs of second and third arches;
pfp palatinal facet for the preethmoid or ethmoid;
php pharyngeal process;
phy pterotic fossa for hyomandibula;
phy' pterotic head of hyomandibula;
pm posterior myodome;
pm' basioccipital part of myodome;
pmx premaxilla;
poe anterior lateral semicircular canal opening in pterotic;
pop preopercular;
ppm palatinal process of maxilla;
ppp prevomer and preethmoid processes of maxilla;
ppq posterior process of quadrate;
ppv posterior process of prevomer;
pre preethmoid;
prm maxillary process towards preethmoid;
pro prootic;
pr4 pleural rib of fourth vertebra;
pt posttemporal;
pte pterotic;
ptp pterotic process;
pv prevomer;
pvp prevomerine process of maxilla.
q quadrate.
r rostral;
ra retroarticular;
rpm rostral process of maxilla;
rpp rostral process of premaxilla;
rps ridge on parasphenoid.
sar sesamoid angular;
sca sensory canal in angular;
scd sensory canal in dentary;
scl supracleithrum;
sco sensory canal in opercular;
se supraethmoid;
sh stylohyal;
shy sphenotic fossa for hyomandibula;
shy' sphenotic head of hyomandibula;
so supraoccipital;
sof occipital canal in frontal;
sop supraoccipital process;
sp sphenotic;
st supratemporal;
stf subtemporal fossa;
suo supraorbital;
sup subopercular;
sy symplectic;
sym symphysis.
t2—t5 pharyngeal teeth;
tc temporal sensory canal;
tfo trigeminofacial orifice;
tp transformator process;
tri tripus.
uoa upper opening for the anterior semicircular canal;
uop upper opening of the posterior semicircular canal.
ixf glossopharyngeal foramen;
xf vagus foramen;
4 trochlear foramen;
5 foramen for main branches of trigeminal;
6 abducens foramen;
7 foramen for branches of facial.

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STUDIES ON THE PHYLOGENY OF BIRDS

BY

NILS HOLMGREN†

During the last year of his life, Professor Nils Holmgren, the founder and first editor of Acta Zoologica, was engaged with a large work on the phylogeny of birds. Before illness overpowered him he had composed the manuscript which is hereby published in accordance with his expressed wish. It consists of three parts (designated below as Parts I, II and III) which deal respectively with the early ontogeny of the limbs and the pectoral and pelvic girdles in birds, the phylogeny of the feathers, and the problem of the phylogeny of birds as a whole.

Professor Holmgren himself considered the first part as complete, the two remaining ones as tentative sketches indicating the lines along which the investigation should proceed though the real research work was only just begun. A certain amount of editorial work has been necessary to make the manuscript ready for publication, but, this has been confined to the formal sides such as the disposition of the paper, the arrangement of the illustrations, the literature references, etc. No attempt has been made to complete the fragmentary portions or to illustrate the chapters lacking pictures. There are several precedents of such amendments to posthumous works being more likely to obscure than to elucidate the author's intentions.

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I. HAND, FOOT, AND PELVIS IN BIRDS — AN ONTOGENETICAL STUDY

1. INTRODUCTION

It is very interesting to follow how the opinions about the homologies of the birds' fingers have fluctuated between the original interpretation of MECKEL (1825) and that of OWEN (1835—36, 1849). According to MECKEL the fingers are the 1st, 2nd and 3rd, according to OWEN they correspond to the 2nd, 3rd, and 4th of the pentadactylous hand. MECKEL does not seem to have given any other evidence for his opinion than that the thumb is generally the 1st finger on the radial side of the hand and that the other two must thus be the 2nd and 3rd. OWEN based his interpretation partly upon a comparison between the anterior and the posterior extremities. He says (p. 73): "In all birds the three metacarpals, here seen to be distinct, coalesce with one another and form a single bone, having an interesting analogy to the metatarsus, which likewise consists in all birds of a coalescence of the three bones supporting the corresponding toes, namely, those answering to the second, third, and fourth in the pentadactyle foot."

MECKEL's idea of the numbering of the fingers in the bird's hand was accepted by most of the great anatomists of the last century i.e. VON BAER (1828), CUVIER (1835), GEGENBAUR (1865), HUXLEY (1868), MILNE-EDWARDS (1872), FÜRBRINGER (1888), GADOW (1891—93), and others. GEGENBAUR seems to have given the theory a new content, comparing the hand of birds with the hand of reptiles, especially that of the crocodiles. In the crocodiles the hand is reduced, with the 4th and 5th fingers a little smaller than the others and with but two elements in the proximal carpal series. These elements he interpreted as "radiale" and "ulnare" and held them to be homologous with the "radiale" and "ulnare" in birds. Nowadays, however, we know that this homology cannot be sustained any longer. Therefore GEGENBAUR's argumentation has lost its actuality. GEGENBAUR pointed out the very great value of embryological statements when determining the homologies, for instance, between organs which are adapted for different purposes. Still, MECKEL's theory was largely destitute of embryological support. But that state of affairs was soon altered. ROSENBERG (1873), who accepted MECKEL's theory, found a rudimentary 4th

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finger in the hand of the chick, but not a 5th, the presence of which would have been decisive for proving the theory. ZEHNTNER (1890) investigated the development of the extremities in *Cypselus melba*. A rudiment of the 4th finger was found to be present there, but no trace of a 5th. W. K. PARKER (1888) and T. J. PARKER (1892) both accepted MECKEL's theory without comment. In *Apteryx* (T. J. PARKER) the 1st and 3rd (2nd and 4th) digits become reduced, but the 2nd (3rd) develops strongly. In W. K. PARKER's paper on the development of the wing of the chick he described a small cartilage lying radially to the 1st (2nd) metacarpale. He suggested that this cartilage is possibly a prepollex. Its position, being so far distad, however, does not make this interpretation very probable but suggests that it is a 1st digit. BROOM (1906) investigated the development of the wing of the ostrich. Four digits were recorded, of which the 4th is rudimentary. He laid stress upon the phalangeal formula (2, 3, 4) of *Archaeopteryx*, which is identical with that of the 1st, 2nd, and 3rd digits in many reptiles.

The most important contribution to the theory of MECKEL was that of STEINER (1922). He studied the development of the hand especially in *Anser anser*. His material was, however, incomplete as the early stages of the hand were taken from duck and chick. The paper is beautifully illustrated with pictures, some of which, however, are combined from a number of sections. These pictures thus cannot be regarded as first class evidence. STEINER says: "Mit dem Nachweis aller dieser Homologien haben wir aber eine wesentliche Bestätigung der durch die embryologischen Ergebnisse nahegelegten Deutungsweise der Fingerstrahlen des Vogelflügels erhalten. Jenen zweiten Weg zur Entscheidung dieser Frage, welcher von vergleichend-morphologischen Gesichtspunkten aus noch verwandt werden könnte, nämlich der Vergleich der Vorderextremität mit der Hinterextremität, glaube ich deshalb nicht mehr betreten zu müssen. Es würde uns zu dem genau gleichen Resultate führen, da die Hinterextremität, mit Einschluss des Vogelfusses, auf dem gleichen Grundplan des Autopodiums, welcher soeben für die Vorderextremität wahrscheinlich gemacht wurde, zurückgeht. Schon embryologisch müsste die Tatsache, dass auch im Fusse der IV Strahl eine beschleunigte Entwicklung aufweist, die gleiche Zählweise der Fingerstrahlen des Vogelflügels ergeben." But if the two methods give different results, which is the most reliable one?

STEINER found two rudimentary rays at the ulnar side of the hand. These rays, he considered, represent the 4th and 5th digits. The functional fingers were accordingly considered to be the 1st, 2nd, and 3rd. However, the "4th" rudiment is the well-known ulnare rudiment and the "5th" is no finger-rudiment at all, but the pisiform part of the ulnare-pisiforme.

In 1934 and 1949 STEINER and in 1942 his pupil LUTZ maintained the chief results of STEINER's investigations of 1922 and made some corrections and addenda. I shall consider them in connection with my own new investigations.

Meanwhile OWEN's theory had also gained supporters from embryological points of view. LEIGHTON (1894) investigated the development of the wing of *Sterna wilsoni*. He came to the following conclusion: "We may conclude, then, that the only conditions possible are either I, II and III, or II, III and IV, and that until some evidence be found of the actual appearance of a fifth digit on the ulnar side, there is at least as much reason for the second as for the first formula." ELISA NORSA (1895) writes: "Sur les coupes, et surtout sur celles qui sont plus proches de la face palmaire du membre, *l'on voit partir de la masse du carpe cinq lobes ou saillies dont les trois médianes sont plus apparentes et correspondent aux trois doigts de l'aile dont elles formeront l'ébauche du squelette*. La saillie situeé vers le bord ulnaire et qui formera le métacarpien rudimentaire décrit par Rosenberg est moin développée: encore moins celle du bord radial. Nous avons observé ce stade chez les poulets de 5 jours et chez les pigeons de 6. Si l'on admet que les 5 lobes en question correspondent aux 5 doigts de la main typique, il faudra en déduire que les trois doigts de l'aile sont ceux qui, dans le membre pentadactyle portent les numéros II, III, IV et adopter la numération de OWEN." This is, as far as I know, the first time that a rudimentary finger has been mentioned on the radial side of the so-called thumb. One objection might perhaps be made to NORSA's interpretation of the most radial lobe, viz. that it might represent a prepollex rudiment: this seems to be STEINER's opinion. SIEGLBAUER (1911) found a similar lobe in *Anas boschas* and *Pygoscelis papua*. He interpreted it as the 1st finger of the pentadactylic hand. These lobes were also seen and depictured by PREIN (1914) in a number of developmental stages of the common domestic fowl. In the 5- and 5½-day stages the rudiment of the 1st finger is not separated from the rest of the tissue at the radial border of the carpus but in the 6-day stage this 1st finger is easily discernible, placed distal to the "radiale." In the 7-day stage the 1st finger rudiment is not visible any longer.

Meanwhile, MEHNERT (1897) had found in a 16-day-old ostrich embryo a large process on the outside of the "1st" metacarpale, which he interpreted as the 1st digit of the pentadactylic hand. He says: "From the ontogeny of the Ostrich wing we learn that the tridactyle hand originates from the pentadactyle by the disappearance of the first and the fifth rays. The persisting digits, then, are 2, 3 and 4 and the longest digit of the wing is not, as hitherto supposed by most authors, the second but the third ray."

In my paper "On the origin of the tetrapod limb" (1933) I published a summary report of an embryological investigation of the wing skeleton in birds. This study includes a comparison between the hand and the foot of a somewhat advanced stage of *Larus canus*. It resulted in the conclusion that the functional fingers are the 2nd, 3rd and 4th. A rudimentary 1st and a 5th finger were described.

Following the development of the wing in the common fowl, MONTAGNA

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(1945) gave a strong evidence for the theory of OWEN. Nevertheless STEINER (1949) says: "Als Hauptflugfinger dient *bekanntlich*[1] der zweite Finger der ursprünglichen pentadactylen Tetrapodenhand."

To this short history of the theories concerning the bird's wing skeleton it may be added that MECKEL's theory regarding the functional fingers as nos. I, II and III has been accepted by most authors of textbooks and manuals, even the most recent. OWEN's theory about the functional fingers as nos. II, III and IV is, as far as I know, only met with in the textbooks of WIEDERSHEIM (1909) and SCHIMKEWITCH (1910) and in LILLIE & HAMILTON's "Development of the chick" (1952).

In spite of all the work concerning the question of the numbering of the bird's fingers and all the discussion about it, the last word has apparently not yet been said on this question. And, yet, it is of basic importance, especially from phylogenetical points of view. One is bound to ask: if the numbering of the fingers of recent birds cannot be satisfactorily determined, how can one expect to be able to determine the numbering of the fingers in, for instance, *Archaeopteryx?* If the fingers of recent birds are the 1st, 2nd and 3rd, they must be likewise in *Archaeopteryx*; if the fingers of the recent birds are the 2nd, 3rd and 4th, then they are the same in *Archaeopteryx*. These two possibilities involve two different lines of descent of the birds: either from reptiles with degenerating 4th and 5th fingers or from reptiles reducing the 1st and 5th fingers.

From these points of view it is necessary to take up the problem once again, especially as the most recent investigators, STEINER and LUTZ, HOLMGREN and MONTAGNA have arrived at quite different results: STEINER and LUTZ in favour of MECKEL's theory, MONTAGNA and HOLMGREN confirming the opinion of OWEN.

2. MATERIAL AND METHODS

My material consists of series of embryos of *Struthio australis, Gallus domesticus, Anser anser, Larus canus* and *L. fuscus, Fulica atra, Podiceps cristatus, Pica pica, Columba livia,* and *Ploceus sp.* The series of *Struthio* contains embryos 9, 10, 11, 12, 13, 14, 15, 18, and 21 days old. These embryos were collected by Mr. G. H. FRANK of the Stellenbosch University and by Professor C. A. DU TOIT also of that University and were presented to me when I visited Stellenbosch in 1949 and 1951. I am much indebted to these gentlemen for their kind generosity. I also owe a very great debt of gratitude to the Swedish Natural Science Research Council and to the University of Stockholm for their generous grants, which enabled me to undertake two embryological expeditions to South Africa.

Besides the ostrich material I had at my disposal an abundance of series of

[1] The italics are the present author's.

Gallus, Anser and *Larus,* which have enabled me to follow the development of the hand and the foot without any gaps in the series. The material was generally fixed in BOUIN's fluid, but sublimate mixtures such as sublimate-formalin-acetic acid were also used. Staining generally in HEIDENHAIN's azan. The muci-carmine recommended by STEINER did not give good results, perhaps owing to bad carmine. HARRIS' hematoxyline staining as modified by MONTAGNA was also used, but did not give satisfactory results.

3. HAND AND FOOT IN EARLY DEVELOPMENTAL STAGES

Struthio australis.

Hand.

Embryo 9 days old (fig. 1). The hand is an oval disc. The humerus is weakly chondrifying. The ulna and radius are present as dense mesenchymatic or prochondrial rods. Together they form an angle of about 60°. The ulna is somewhat longer than the radius. The distal end of the radius disappears in a mesenchyme, which is denser than the mesenchyme between the ulna and the radius. The radial mesenchyme (at the tip of the radius) has no distinct outline. The ulna has a short distal mesenchymatic continuation. This continuation is the rudiment of the *ulnare.* On its tip it carries the rudiment of the most ulnar part of the carpal arch. This rudiment is a separate rounded body. As in all other tetrapods, the carpal arch with the digits (neopodium) is in birds also formed distally to the archepodium from rudiments of its own. On the distal surface of the carpal rudiment a diffuse rudiment of a finger is already appearing. There is also a diffuse continuation of the carpal element in a radial direction. This continuation (not seen in fig. 1) forms the carpal arch of this stage. It nearly reaches the radial blastema.

In trying to determine which is the first appearing finger of the bird's hand we must stress the fact that this finger develops at the tip of the ulnar "Randstrahl" and therefore is certainly the 4th finger. This conclusion is quite in accordance with the conditions in reptiles and mammals. STEINER (1922) emphasized this relation between the 4th finger and the ulnare.

Thus the first ulnar rudiments to appear in Struthio are the ulna, the ulnare, the "carpale" of the 4th finger and the rudiment of the 4th finger. If there is any doubt about this conclusion, it will be removed when following the further development of the hand.

Embryo 10 days old. Here the development has not advanced very far from the preceding stage. However, on the "carpale" of the 4th finger a small ulnar outgrowth indicates the development of a finger ulnar to the 4th (fig. 2). *This*

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Fig. 1 *Struthio australis.* 9 days. Hand.

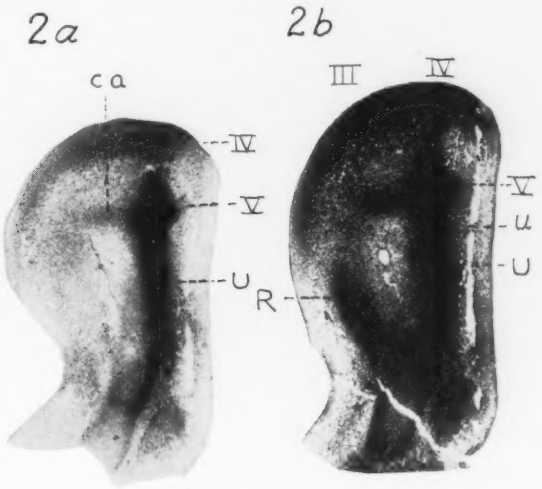

Fig. 2. *Struthio australis.* 10 days. Hand.

finger rudiment must thus be the 5th. As the 4th as well as the 5th finger rudiments are inserted into the "carpale", this "carpale" may be a 4th and may perhaps also include a 5th carpale, but these parts are not differentiated from one another, neither is carpale 4 differentiated from the continuation of the carpal arch in the radial direction. In the 10-day stage the carpal arch is fairly strongly developed at right angles to the ulna and ulnare. The tip of the arch reaches the tip of the radial blastema, where it seems to merge into it.

In another embryo (fig. 3), labelled 10 days old, the hand has developed a small stage further. Apically to the carpal arch and between the 4th finger and the radial end of the arch *the rudiment of the 3rd finger is appearing.* It constitutes a separate blastema. In the "carpale" of the preceding stage a prochondrium is developing proximal to it. This prochondrium is the differentiating ulnare.

Another embryo said to be 10 days old and measuring 15 mm. is somewhat more advanced than the preceding one. The rudiments of the 5th, 4th and 3rd fingers are fairly well developed (fig. 4). The 5th rudiment is inserted into the lateral part of the apical surface of the ulnare, whereas the 4th digit with its carpale occupies the very tip of the same. The carpal arch is broadly joined to the radial blastema. About where the arch and the blastema meet, the still indistinctly outlined rudiment of the 2nd finger is discernible.

In the previously described 9- and 10-day stages the space between the ulna and ulnare and the radial blastema is "empty", i.e. occupied by a sparse mesenchyme generally devoid of special differentiations. Now this space contains a rather dense blastema (central blastema) extending from the ulnare and the

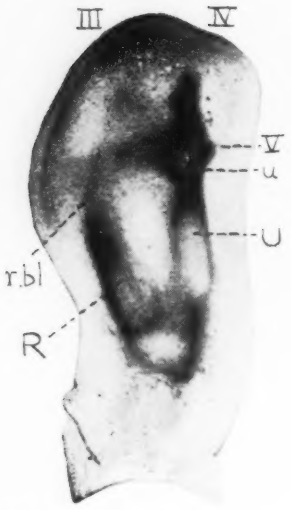

Fig. 3. *Struthio australis*. 10 days. Hand.

Fig. 4. *Struthio australis*. 15 mm (10 days?). Hand.

apical end of the ulna to the blastema proximal to the rudiment of the 2nd finger. Another blastema, not quite so dense, is found on the ulnar side of the apical part of the radius. The future of the two parts of the central blastema will be considered further on. Here it needs only be added that the central blastema is not delimited from the carpal arch.

Foot.

Embryo 9 days old. The feet in birds generally develop earlier than the hands. The feet of a 9-day ostrich embryo thus correspond in their development to a 10-day stage of the hand, in which three finger rudiments are present. In the foot the fibular border ray corresponds perfectly to the ulnar border ray in the hand. The fibula runs distally out in the fibulare and the 4th toe rudiment (fig. 5). In addition the 5th toe is inserted into the fibular surface of the fibulare. The carpal arch is a strong transversal band which tibially borders on the tibial blastema. The 3rd toe has developed at the tarsal arch. So far the foot of the 9-day ostrich is identical with the hand of a 10-day embryo. But to these features is added a strong mesenchymatic or prochondrial transversal bridge joining the fibulare with the tibial blastema. This bridge is the central blastema. It is well separated from the tarsal arch. Had this 9-day embryo been but "a hair's breadth" younger, the central blastema would not yet have existed, and the agreement would then have been perfect. But as this 9-day foot is developed, it agrees almost exactly with the hand of the 15 mm. ostrich embryo. The only

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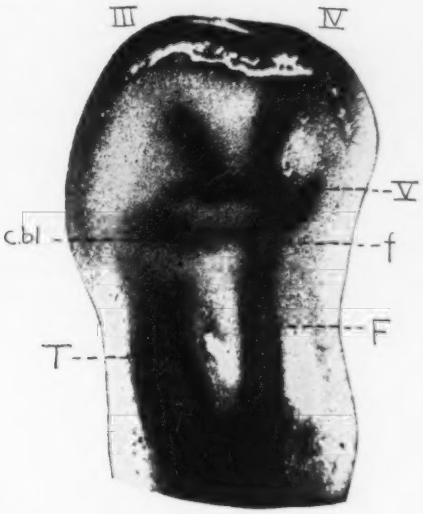

Fig. 5. *Struthio australis.* 9 days. Foot.

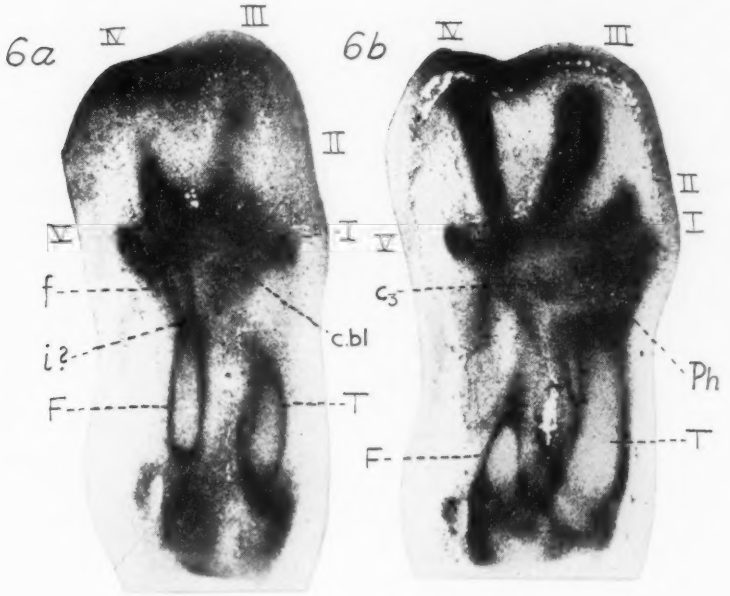

Fig. 6. *Struthio australis.* 10 days. Foot.

difference seems to be that there is no empty space between the carpal arch and the central blastema, these two structures being confluent in the hand. As will be mentioned further on, the central blastema is already differentiating.

Embryo 10 days old (fig. 6 a, b). In this stage all five toes are developing.

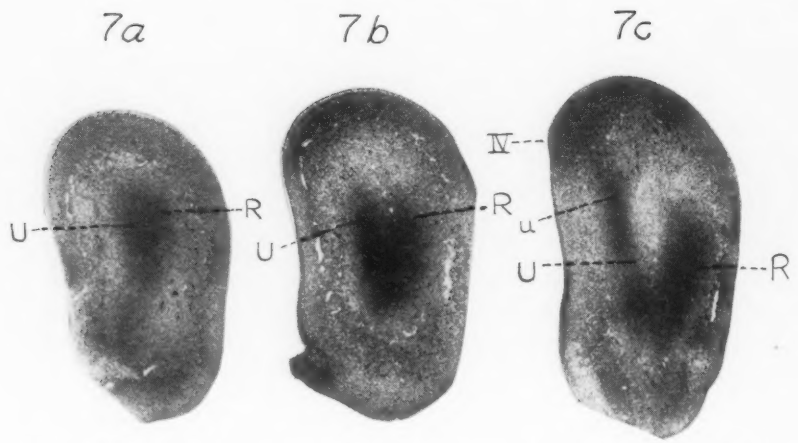

Fig. 7. *Anser anser.* (a) 6 days, (b) 6 days 4 hours, (c) 6 days 12 hours. Hand.

The fibula and the fibulare as well as the tibia are chondrified and the central blastema is chondrifying as a band between the fibulare and the tibial blastema. The tarsal arch, still prochondrial, is about to chondrify. In this stage it is possible to observe that the 2nd toe develops at the tibial end of the tarsal arch. It is also easily observable that the rudiment of the 1st toe develops just at the tibial end of the central blastema, tibially to the chondrifying "tibiale" (c 2).

In this stage as well as in the preceding one the tibia ends distally with a triangular portion, one tip of which is directed disto-tibially. This triangular part represents the tibiale with the prehallux (fig. 6 a, Ph).

Anser anser.

Hand.

Embryo 6 days old (fig. 7 a). The rudiment of the hand skeleton is very little advanced. The humerus and the cubital bones are represented by a single large blastema. This blastema contains no perforating blood-vessels (interosseal vessels). Such vessels are, however, found in front of the blastema. They run dorsad from a ventral blood-plexus.

Embryo 6 days 4 hours (fig. 7 b). The single blastema of the preceding stage has increased frontad. By this process of growth blood vessels previously situated in front of the blastema have become enclosed in it as it has extended frontad, cleaving it into two branches, one on each side of the blood vessels at the tip of the original blastema. The two branches are the rudiments of the ulna and the radius.

Embryo 6 days 12 hours (fig. 7 c). The ulna and the radius are now represented by two mesenchymatic rods, diverging slightly frontad. The ulna is

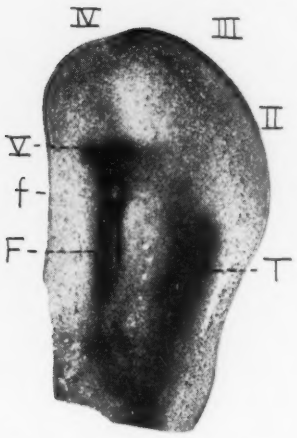

Fig. 8. *Anser anser*. 6 days 13½ hours. Foot.

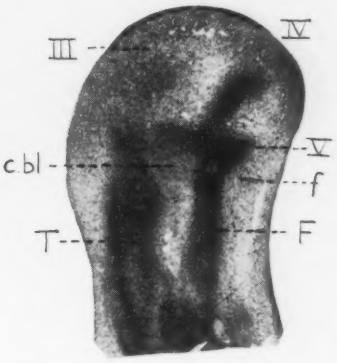

Fig. 9. *Anser anser*. 7 days. Foot.

slightly longer than the radius, but is continued towards the tip of the limb by a blastemic column, somewhat denser than the common blastema of the limb. This blastematic column is the rudiment of the ulnare and perhaps also of the "carpale" and the 4th finger. Radius merges frontally with a somewhat broad blastematic field without any differentiation.

Embryo 6 days 13½ hours. This embryo differs from the preceding one only in the development of the ulnar branch. The obscurely outlined pillar of blastema at the tip of the ulna is now very pronounced, though still without differentiations.

Embryo 7 days old. In this embryo the ulnar branch is differentiated. Basally the ulna is about to become prochondrial. Apically to this part there follows a somewhat narrower portion, which is still a blastema. This portion represents the ulnare. It merges apically with a short, thick portion extending into the frontal end of the limb. This portion is the "carpale." On its apical end it seems to carry a very indistinct finger rudiment. On its ulnar side there is a suggestion of another finger rudiment.

Foot.

As in the ostrich, the development of the foot in *Anser* is at each early age in advance of that of the hand. In *Anser* the difference is about 12 hours.

Embryo 6 days old. The foot of a 6-day embryo does not differ at all from the hand of an embryo of 6 days 12 or 13½ hours. The fibular stem is about to differentiate in the same manner as the ulnar stem in the hand.

Embryo 6 days 4 hours. In this embryo the development of the foot has advanced as far as has the hand of a 7-day embryo. The fibular stem is dif-

Fig. 10. *Gallus domesticus*. 4 days 18 hours. Hand.

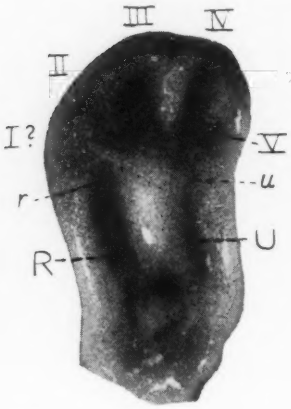

Fig. 11. *Gallus domesticus*. 5 days. Hand.

Fig. 12. *Gallus domesticus*. 5 days 10 hours. Hand.

ferentiated. Rudiments of two toes, the 4th and the 5th, are obscurely seen at the tip of the fibular column. The radial blastema is about to become connected with the "carpale", a carpal arch forming.

Embryo 6 days, 12 hours. At this stage toes 4 and 5 are more clearly visible, though still not sufficiently distinctly outlined to permit of a definite statement as to their configuration. The tarsal arch is also indistinct. The tibial blastema seems to increase, later to join the tarsal arch. But all these structures are still indistinct.

Embryos 6 days 13½ hours (fig. 8) *and 7 days* (fig. 9). These two stages agree very closely with one another. In the later of them, however, fibula and tibia are chondrifying. The fibular stem is differentiated with a prochondrial fibulare on the tip of fibula. The tarsal of the 4th toe is inserted in the apical end of the fibulare. The 5th toe is situated on the fibular side of tarsale 4. The tibial arch runs vertical to the fibular branch and joins the tibial blastema. In addition to the 5th and 4th toes the 3rd is appearing as a very indistinct blastema apical to the tarsal arch. The 2nd and 1st toes are still not present.

This stage (fig. 8) of the development of the foot in *Anser* is nearly identical with that of the hand of a 10-day ostrich (fig. 3), but a little less advanced than the foot of a 9-day ostrich (fig. 5), which is almost identical with the 7-day stage of *Anser* (fig. 9).

Gallus domesticus.

Hand.

The early development of the limbs in *Gallus* is not at all favourable for study as it is concentrated within less than 24 hours.

Embryo 4 days 6 hours old. In a 4-day embryo the hand and the foot skeletons are not present at all, but six hours later the ulna and radius are well developed. The ulna is extending further frontad than the radius. The rudiment of the ulnar stem ends frontally with a somewhat widened short and fairly diffuse portion: "carpale". No finger rudiments were discernible on this "carpale".

Embryo 4 days 12 hours. The hand at this stage has not advanced markedly as compared with the preceding stage. The carpale 4 is a little more pronounced, but no finger rudiments have appeared as yet.

Embryo 4 days 18 hours (fig. 10). The hand skeleton is a little further advanced and the rudiments of the ulnar parts have developed so much that the rudiment of the 4th finger has been added to carpale 4. There is a suggestion of a radial extension of the carpale.

Embryo 5 days. In one 5-day hand the 5th finger is still not present. The carpal arch is forming. It issues from the carpale of the 4th finger and is directed somewhat obliquely distad. No rudiment of the 3rd finger is to be seen. Another 5-day hand is more advanced (fig. 11), the 5th finger having appeared on the ulnar side of the carpale 4. The carpal arch extends apically to the radial blastema. It carries the rudiment of the 3rd finger. The 2nd finger is suggested at the radial end of the carpal arch. Between the radial end of the carpal arch and the radial blastema there is a narrow space, which is poor in cells. In a third hand the 5th finger is present, but the 3rd is still a separate rudiment distal to the carpal arch.

Embryo 5 days 10 hours. Three well-differentiated fingers are present. The chondrification is far advanced in the ulna and radius. The "radial blastema" is confluent with the carpal arch (fig. 12).

As will be demonstrated later on, this blastema contains the rudiments of the 2nd and 1st fingers as well as the centrale 2. But these parts are not yet so differentiated that their boundaries could be made out. A central blastema is present between the "radial blastema" and the rudiment of the ulnare. It is confluent with the carpal arch, otherwise being rather indistinctly outlined.

Foot.

Embryo 4 days 6 hours old. There is no noticeable difference between the foot at this early stage of development and the hand of a 4 days 12 hours embryo. The fibular and the tibial border-rays are developed just like the ulnar and the radial ones respectively. The tibial rudiment, however, is broader than the fibular rudiment.

Embryo 4 days 12 hours. The fibular border-ray is differentiating. A tarsale is distinctly delimited from a fibulare portion distal to the fibula. From the "tarsale" the 4th toe issues. The 5th toe has not yet appeared. The rudiment of the tarsal arch and the 3rd toe are forming (fig. 13). Between the tibial end of the tarsal arch and the tibial blastema is an empty space. This stage of develop-

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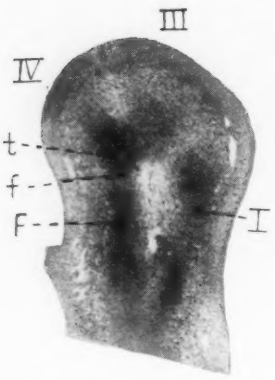

Fig. 13. *Gallus domesticus.* 4 days 12 hours. Foot.

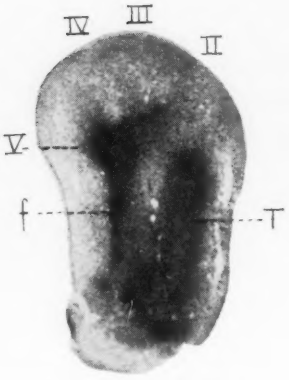

Fig. 14. *Gallus domesticus.* 4 days 18 hours. Foot.

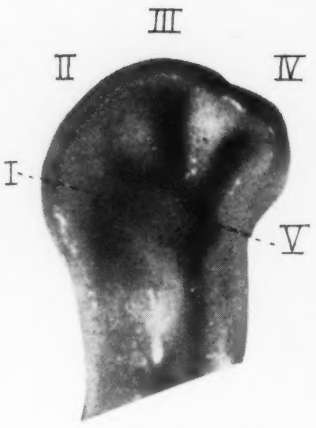

Fig. 15. *Gallus domesticus.* 5 days. Foot.

ment of the foot corresponds closely to an early 5-day stage of the hand (fig. 11), as described above.

Embryo 4 days 18 hours. The foot at this stage corresponds closely to the foot at the preceding stage, but the 5th toe is developing. The 2nd toe is also about to form (fig. 14).

Embryo 5 days (fig. 15). In the 5-day stage the 2nd to the 5th toe are developed, but the 1st toe is only diffusely outlined and not in any way delimited from the tibial blastema. The tarsal arch is not separated from the central blastema. The latter is proximally delimited by a line running from the tibial corner of the fibula to the tibial blastema.

Embryo 5 days 4 hours. All five toes are now present. The 1st toe is less developed than the 2nd, the 3rd and 4th toes are the most consolidated ones. The 1st toe is clearly seen to be inserted in the rudiment of the centrale 2 ("tibiale"). Tibia and fibula are chondrified.

Statements and conclusions.

(1) It has been stated that the rudiments of the fingers (and probably also of the toes) appear distal to the stem portions of the hand as mesenchyme condensations originally separated from the stem. This statement is supported by observations of the 9-day stage in *Struthio* and the 9 mm. stage in *Larus,* where the carpale 4 is a separate structure. In the other early stages described in the preceding pages this carpale (tarsale) has already joined the ulnar (fibular) branch of the stem. There is thus in birds a clear distinction between the archepodium and the neopodium, as there is in other tetrapods. The carpale (tarsale)

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4 probably contains the prospective rudiments of the 4th and 5th fingers (toes). The carpale (tarsale) 3 appears as a process on the radial side of the carpale 4 or as a separate rudiment in that position. The 3rd digit generally has a separate origin distally to its carpale (tarsale) rudiment. The carpale (tarsale) 3 and its digit may, however, originate together (as for instance in the chick). The carpale (tarsale) 2 arises at the radial end of the carpal (tarsal) arch. The 1st toe develops together with the tibial blastema. In the hand the corresponding finger is rudimentary. The future carpalia (tarsalia) together form a transversal continuous band of condensed mesenchyme (or prochondrium): the carpal (tarsal) arch.

(2) It is stated that *with every stage in the early development of the hand there corresponds an almost identical stage in the foot.* This is a statement which enables us to carry out a detailed comparison between hand and foot.

(3) From the statements made in the preceding paragraphs it follows that a comparison between the hand and the foot is not only possible but highly recommendable for the interpretation of the hand in birds.

In the foot the fibula and the fibular branch run out distally in the 4th toe. On the fibular side of its tarsal element a 5th toe develops somewhat later. In the hand the ulnar branch runs out in a digit, the carpale of which carries somewhat later a small ulnar finger rudiment. *Since the foot of the birds at a certain stage always has 5 toes, the determination of the two fibular toes as the 4th and 5th is immediately settled. The comparison with the hand thus immediately results in the inevitable conclusion that the two ulnar fingers are the 4th and the 5th.* From this conclusion it follows that the missing finger in the bird's hand is the 1st. The 5th finger subsequently disappears, and thus *the functional fingers of the bird's hand are the 2nd, 3rd and 4th and not the 1st, 2nd and 3rd.*

(4) In the early development of the hand in birds there is no ulnar abduction of the ulnar "Randstrahl". Such an abduction appears much later when the head of the ulna bends radiad, causing the ulnare and the 4th and 5th fingers (STEINER's 3rd and 4th) to form an angle with the forearm. The early "ulnar abduction" of which STEINER speaks is due to his mistaking the 5th finger for the 4th (see further on). The abduction of the 5th finger is the normal feature of the tetrapod hand.

Discussion.

In spite of the fairly large number of authors who have treated the development of the hand and foot of birds, it is a remarkable fact that the early development has been largely neglected. Indeed, the only exceptions are PREIN (1914), who has depictured sections through the hand of two early stages of *Gallus domesticus*, and STEINER (1922), who has described two early stages of the wing development in *Anas boschas* and one not quite so early stage of *Gallus*

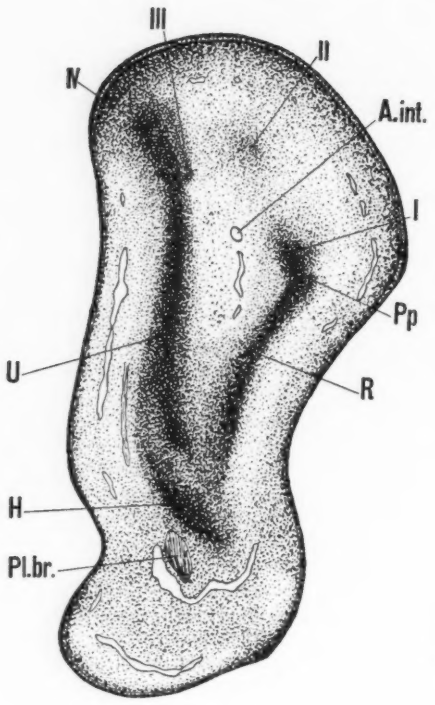

Fig. 16. "Flügelanlage eines 6¼ tägigen Embryos von Anas boschas. (Kombinationsbild aus mehreren Schnitten.)" After STEINER 1922, fig. 2.

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domesticus. The early development of the foot of birds seems to be quite unknown, and this is the reason why the almost p "fect agreement between the hand and the foot of very young embryos has escaped the embryologists.

The hand of a 6-day embryo of *Anas boschas* described by STEINER (1922) is a little more advanced than the 6 days 4 hours embryo of *Anser anser* shown here in fig. 7 b, and a little less advanced than that of 6 days 12 hours shown in fig. 7 c. STEINER's picture of this 6-day hand is from a single section, his other pictures, on the contrary, are produced by combining several sections. This first picture thus possesses a more objective value than the others. This becomes perfectly clear when we consider STEINER's reconstruction of the hand of a 6¼-day-old *Anas boschas*. His picture cannot be compared with any of the many series of sections through the hand of bird embryos at a corresponding stage of development in my collection. The following objections may be made to fig. 2 in STEINER's paper (fig. 16).

(1) In my material I have never found fingers "4" and "3" (STEINER's numbering) parallel to one another and more or less confluent longitudinally. They are always strongly divergent (the "4th" generally perpendicular to the "3rd").(2) STEINER stressed that in Sauropsids the ulna-ulnare and the"4th" finger form a straight line. In all my young bird embryos the ulna-ulnare runs out

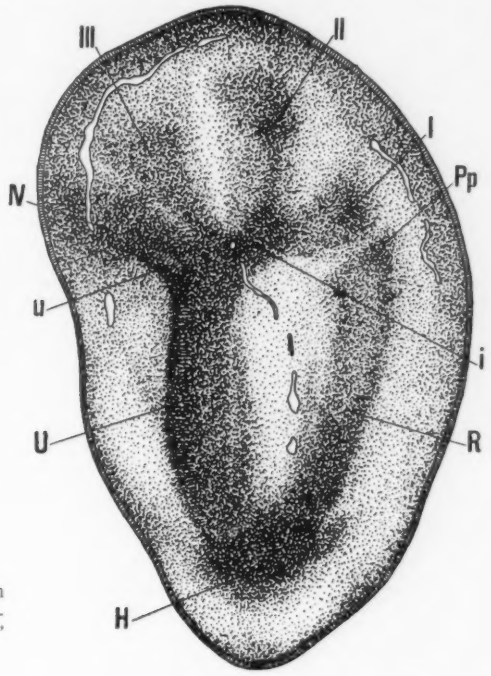

Fig. 17. "Flügelanlage eines 5¼ tägigen Embryos von Gallus domesticus. (Kombinationsbild aus mehreren Schnitten.)" After STEINER 1922, fig. 3.

directly into a finger rudiment. As there is no ulnar abduction of the hand in early stages of development, this finger is the 4th. On the ulnar side of this 4th finger a rudiment of a 5th finger appears, perpendicular to the base of the 4th. In *Anas* where STEINER has chosen the ulnar one of the two parallel finger rudiments to be the 4th, there is no such perpendicular rudiment present. In *Anas* fingers "4" and "3" both lie in line with the ulna-ulnare, and the choice of the radial one of them to be the 4th finger is amply justified. In that case his "4" can represent the 5th according to my description. (3) The 2nd finger in *Anas* appears in the same position as the 3rd in my material. The 2nd finger in my material appears invariably between this 3rd and the "radial blastema", at the radial end of the carpal arch. This very conspicuous structure seems to have escaped STEINER's attention as he labels it "intermedium" in the 5¼-day chick. (4) There must be considerable doubt regarding the *early* ulnar abduction of the hand as I have never met with such an abduction in my extensive material. An ulnar abduction, undoubtedly present in bird embryos, arises much later and will be considered later on.

The hand of the 5¼-day chick described by STEINER corresponds closely to the hand of my 5-day embryo (fig. 11) and also to the 5-day embryos studied by PREIN and by MONTAGNA. There are, however, some remarks to be made concerning STEINER's interpretation of this hand (fig. 17). (1) The "intermedium"

of this stage is certainly no intermedium at all but the — in most birds very thick — carpal arch, which belongs to the neopodium, and from which the series of carpalia arise. In the 5—5¼-days' stage the true intermedium and the complex of centralia have not yet appeared (MONTAGNA's interpretation will be considered later on). (2) The ulnare of STEINER's description is the densest part of the mesenchyme of the 5th finger. The rudiment of this finger from the very outset stains very strongly with chondromucoid stains. It is this rudiment which has been taken for the ulnare. As this "ulnare" is said to represent the "4th" finger, which has become abduced to the ulnar side, some light is thrown upon the theory of the *early* ulnar abduction. (3) A comparison with my picture of the 5 days 10 hours stage of *Gallus* shows that the radius-prepollex of STEINER's description also includes a sizable radiale.

On the 5¼-day stage of *Gallus* STEINER writes (p. 316): "Dieses soeben geschilderte und in Fig. 3 abgebildete Entwickelungsstadium ist von allen ontogenetischen Stufen des Vogelflügels eines der bedeutsamsten, soweit ein Vergleich mit der embryonalen Anlage der Extremitäten anderer Tetrapoden in Betracht fällt." — — "Es zeigt nämlich dieses Stadium eine ganz überraschende Ähnlichkeit mit dem Aufbau der Extremität der niedersten Tetrapoden, speciell der Urodelen. Mit dem Hinweis auf die von mir andernorts (1921) gegebene Begründung meiner Auffassung über die Zusammensetzung speciell des Autopodiums der Urodelen kann ich mich hier mit der Erwähnung der folgenden wichtigsten Verhältnisse begnügen: 1. Auch in der Extremität der niedersten Tetrapoden endigt der radiale Randstrahl stets nur in einer einzigen kurzen Fingeranlage, welche den sogenannten Präpollex darstellt (EMERY, 1898). 2. Von der Anlage des eigentlichen I. Fingers ist der radiale Randstrahl stets deutlich getrennt. 3. Die Anlage des I. und II. Fingers konvergiert proximalwärts auf das sogenannte Intermedium zu, welches selbst wieder vom ulnaren Randstrahl nach vorn abzweigt. 4. Der ulnare Randstrahl scheint über das Ulnare sich direkt in den IV. Finger fortzusetzen."—"Wenn wir mit diesen Zuständen den embryonalen Vogelflügeln vergleichen so sehen wir, dass die Verhältnisse bis in alle Enzelheiten in ihm die nämlichen geblieben sind."

The 1st, 2nd, and 4th of these paragraphs are common to all tetrapod hands, and I accept them also as agreeing with the hand of the 5¼-day chick. The 3rd paragraph must, however, be rejected as the intermedium of the said bird embryo is no intermedium at all but the carpal arch; the intermedium develops later, in connection with the radial distal corner of the ulna. (Compare the "intermedium" in STEINER's figs. 3 and 4). With this statement the conclusion that the two fingers at the end of this "intermedium" are the 1st and 2nd fingers, as in Urodeles, is stated to lack any support.

STEINER has concluded "dass die als I. Finger angesehene Anlage tatsächlich das Rudiment des Präpollex darstellt." This conclusion was certainly justified

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as long as the skeletal element distal to the radius was considered to be the radiale. But it was shown to be very probable (HOLMGREN 1933) that this skeletal element is a centrale, an interpretation accepted by STEINER (1934). A finger-like element placed upon a centrale cannot be a prepollex, which, when present, is always placed on the radiale. The cited conclusion of STEINER concerning the 1st finger described by various authors is thus no longer valid.

I may seem to have bestowed undue interest on the early stages described by STEINER; but that was necessary as these stages constitute the chief reasons why STEINER concluded that the functional fingers of the birds are the 1st, 2nd and 3rd and not the 2nd, 3rd and 4th. The critical remarks about the interpretation of these stages make conclusions based upon it very doubtful. As STEINER found no finger rudiment radial to the 1st functional finger, he had to demonstrate the presence of a 5th finger ulnar to the 4th. This he believed he had found in the anterior process of the pisiforme. This question, however, will be discussed further on.

4. THE HAND AND FOOT IN LATER DEVELOPMENT

a. Carpus

Struthio australis.

The first rudiment of the central part of the carpus was found in a *15 mm. embryo* (fig. 4). It may have been about 10 days old. The fingers — the 3rd to the 5th — are clearly seen, and there is also a distinct condensation for the 2nd and possibly another for the 1st finger. On the ulnar side the ulnare is chondrifying. From the distal end of the ulna to the distal end of the radius there extends a blastema which fills up the space between the ulnare, the carpal arch and the radial blastema. The central blastema fits closely on to the carpal arch and no line of demarcation is descernible between them. In the radial blastema the so-called "radiale" is about to be outlined.

There is a gap between the preceding stage and the now following *11-day stage* (fig. 18), but this gap is not so wide as to preclude our forming an idea as to how the development has advanced during the intervening period: The 5th, 4th and 3rd fingers are well developed, though the 5th is already in process of reducing, staining strongly, very narrow. The 2nd finger is still a blastema and the 1st very diffuse. In this stage the carpus is chondrifying. The ulnare is well outlined. The long but narrow 5th finger is inserted in its ulnar surface. A not yet prochondrial intermedium is present at the radial corner of the ulna. The rest of the carpus is a single piece of procartilage. The rudiment of the carpal arch and the central blastema together form an oblong piece of procar-

tilage, which by degrees becomes transformed into a single large cartilage articulating especially with the radius but also, by means of the intermedium, with the ulna.

Already in *11-day embryos* the chondrification is well advanced. Fingers 2—5 are chondrified or chondrifying. The 1st finger is visible as a weak rudiment on the radial border of the central mass, just proximal to the basis of the 2nd finger.

The early 11—12 day stages (fig. 18) are interesting as they *show a distinct "prepollex" simultaneously with the rudiment of the 1st finger, which thus cannot be mistaken for a prepollex,* as is done by STEINER.

The pisiforme develops, as usual, below and somewhat laterally to the ulnare. There is nothing unusual about this pisiforme and there is no reason for considering it to be a metacarpale 5 as the 5th digit is inserted in the ulnare in the usual way. Nor is there any justification for the hypothesis that the pisiforme is a pisiforme + carpale 5 + metacarpale 5 (LUTZ 1942). But this question will be discussed fully later on. In the pisiforme are inserted caudally the m. flexor carpi ulnaris and apically the ligamentum piso-metacarpeum (5).

The 13- and 14-day embryos do not differ from the 12-day embryos except in the more mature cartilage of which the carpus is composed. The ventroradial part of the big central blastema has acquired a more distinct outline than in the earlier stages and is — in the 14-day stage — about to become delimited from the carpal complex ventromedially. Intimately connected with the radial corner of the radius there is a rudiment apparently representing a rudimentary radiale (+ prepollex?). This rudiment is still present in a 21-day embryo. As a radiale is thus present, the radial blastema obviously cannot be a radiale, as STEINER (1922) believed, but must represent a centrale (compare HOLMGREN 1933 and STEINER 1934). As will be clear further on, this centrale is probably composed of a medial centrale 1 and a lateral centrale 2. In the 15-day embryo the centrale 1 + 2 is about to become separated from the carpale cartilage. The outline of the distal end of the ulna becomes diffuse as cells seem to be pushed out from it. A considerable group of such cells lies at the radial corner of the ulna and represents the intermedium. Cells originating from the central and ulnar surface of the head of the ulna correspond to the centrale 3 of other birds. Part of these cells lie between the ulna and the ulnare. Some of them later on form the strong ligament that joins centrale 1 with the head of the ulna, others the ligament joining the head of the ulna with the pisiforme. Both ligaments together probably constitute the big ligamentum carpi internum in other birds.

In the preceding lines it has been mentioned that the big radiale centrale in a 14-day stage is about to become separated from the carpal cartilage. In *the 15-day stage* the centrale is perfectly free, but its medioventral outline is indistinct as it appears as if the centrale, when freeing itself from the carpale, had been drawn out, a prochondrial (or slightly condrified) appendix or mass being

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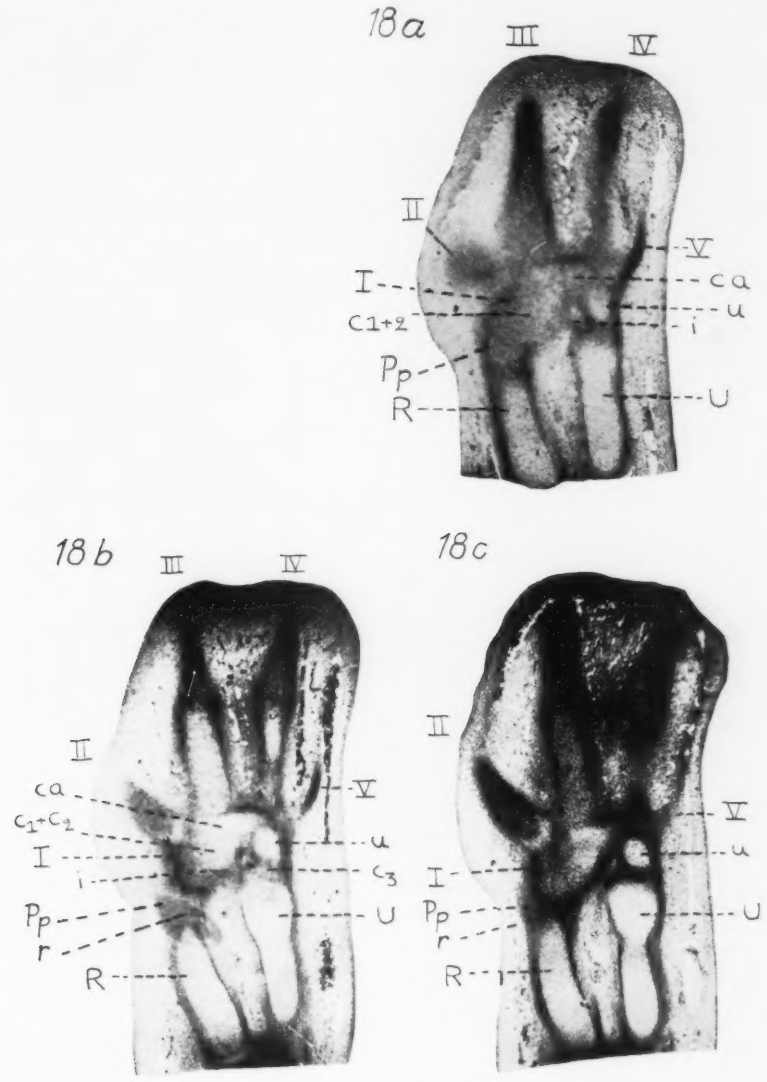

Fig. 18. *Struthio australis.* (a) 11 days, (b) and (c) 12 days. Hand.

formed on its medial surface. This prochondrial mass may possibly represent the centrale 1 portion of the radiale centrale.

In the 18-day stage it is fairly clear that the appendix just mentioned has become incorporated with the radiale centrale, forming a distinct process on its medioproximal end. This process differs structurally from the rest of the centrale, the chondrification being less advanced in the process. Owing to the

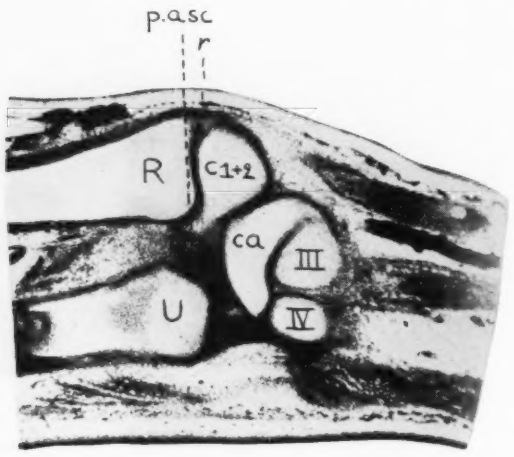

Fig. 19. *Struthio australis*. 18 days. Hand.

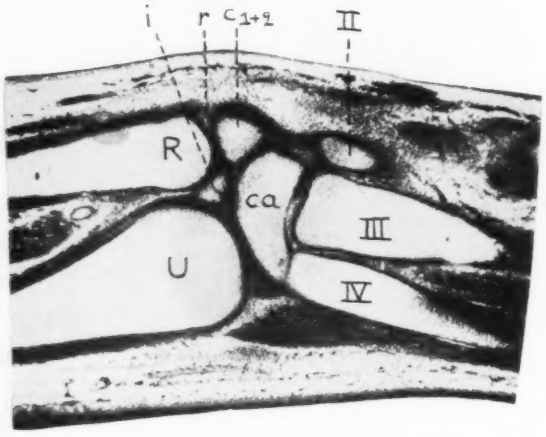

Fig. 20. *Struthio australis*. 18 days. Hand.

very strong development, especially of the carpale 3 portion of the carpal cartilage, the process (appendix of the radiale centrale) has been pressed proximad so that it partially enters in between the heads of radius and ulna (fig. 19). Its tip is now joined to the radius by a ligament. The process in question belongs apparently to centrale 1. The 18-day stage is remarkable because of the appearance of a large, well-chondrified intermedium (fig. 20). Its proximal part lies between the heads of ulna and radius on a somewhat higher level than the centrale 1 process. It extends distad in a distoradial direction. Its proximal part is connected with the head of the ulna by a ligament. From the distal portion of centrale 1 a band extends to the head of the ulna. This band, however, is not very distinct. It seems very probable that it originates from those distal parts of the ulna (centrale 3) which are not well outlined in the 14-day

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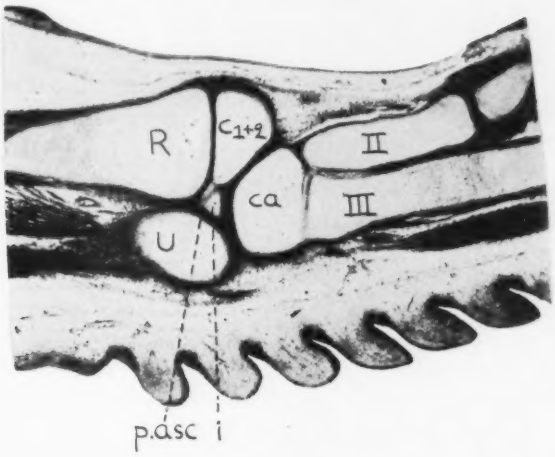

Fig. 21. *Struthio australis*. 19 days. Hand.

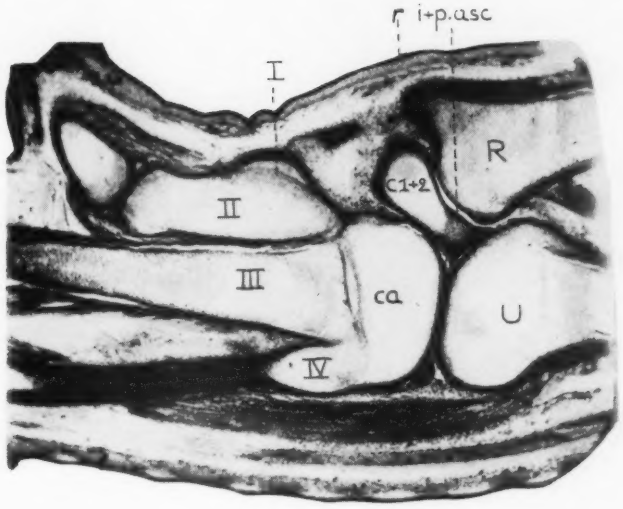

Fig. 22. *Struthio australis*. 21 days. Hand.

stage. An indistinct ligament joining the (ulnare +) pisiforme and the ulna represents the cell group in the 14-day stage which is located between the ulna and the ulnare. The behaviour of these bands will be mentioned in connection with the 21-day stage. The ulnare is about to disappear. The rather small pisiforme is joined by a fine tendon (ligamentum piso-metacarpeum) to the degenerating rudiment of the 5th finger. This 18-day ostrich embryo is the only bird's embryo or bird, excepting perhaps the *Opisthocomus* embryo and *Archaeornis*, that exhibits a well developed (chondrified) independent intermedium.

In an embryo about 19 days old the intermedium is seen to join the upper surface of the centrale 1 process (fig. 21), and in the 21-day stage it has fused

with its dorsal surface but is still not fully fused with centrale 2, a boundary line still existing between centrale 2 and the intermedium (fig. 22), but not between the intermedium and the centrale 1 process. This compound process corresponds to the ascending process of the foot.

In the 21-day embryo the ulnare has disappeared completely; nevertheless, the pisiforme is not very strongly developed — on the contrary it is rather small. In the 18-day embryo the abduction of the hand is an ulnar one, but in the 19-day and 21-day embryos the abduction is a radial one. The abduction is also radial in a more than half-grown embryo. See the picture after C. W. BEEBE in Heilmann (1926). Later on it changes into ulnar again.

In the 21-day embryo the main ligaments of the carpus could be studied. The intermedium is connected with the radial part of the ulnar head (primary intermedium connection). The tip of the process of the centrale is connected with the ulnar side of the radius. From a point slightly distal to the intermedium connection there issues from the boundary part between centralia 1 and 2 a strong ligament in an ulnar direction. It becomes inserted in about the middle of the ulnar head. Another ligament issues from the dorsal part of the pisiforme and becomes inserted also distally in the head of the ulna. (Fibres from the first of these ligaments may possibly connect up with the carpale 3.) The anterior part of the pisiforme is connected by a strong ligament to the metacarpal cartilage and also to the rudimentary 5th finger. This ligament is the lig. pisometacarpeum 5. The fibres which run to the 5th finger form a very distinct separate bundle.

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Anser anser (figs. 23—27).

I have studied a series of 18 embryonic stages of *Anser anser*, ranging from 6 to 10 days of incubation[1]. Generally my results agree fairly well with those obtained by STEINER. There are, however, some points in which our observations do not agree as well as might be desirable. Some remarks and statements are therefore necessary.

(1) In his reconstruction fig. 4 STEINER illustrates a prepollex in a 6½-day stage, located on the radial corner of the radius. In my material the prepollex is never so distinctly outlined as in this figure. In fig. 5 showing a 7½-day stage a prepollex is also illustrated; it is placed distally on the big "radiale" located between radius and the carpal arch. But as we now know that this radiale is a centrale (centrale 2), it is obvious that this latter "prepollex" is not the prepollex of fig. 4, *but must represent the true pollex*, or at least its carpale. In the 8½-day stage the location of the pollex is in the angle between centrale 2 ("radiale") and carpale 2 ("carpale 1") and there is in STEINER's fig. 6 and in my material a blastema (fig. 23) which in my material represents the pollex.

[1] My stages are throughout somewhat less advanced than those described by STEINER (1922).

Fig. 23. *Anser anser*. 7 days 5 hours. Hand.

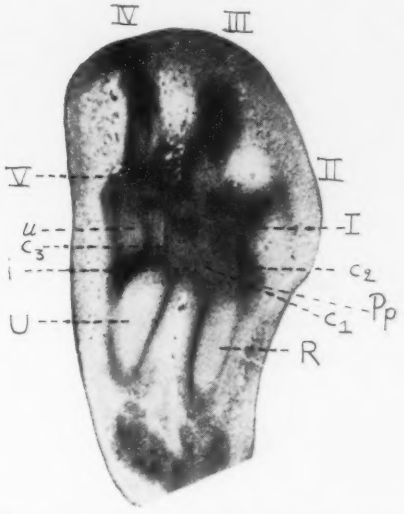

Fig. 24. *Anser anser*. 7 days 15 hours. Hand.

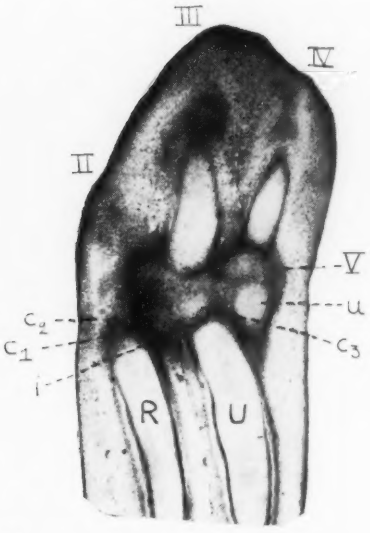

Fig. 25. *Anser anser*. 7 days 22 hours. Hand.

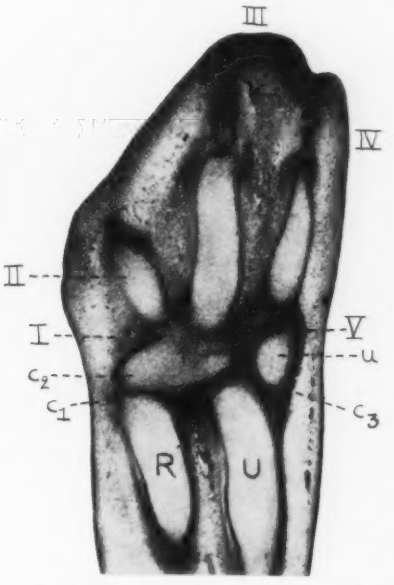

Fig. 26. *Anser anser*. 8 days 4 hours. Hand.

There is no prepollex present. In *Anser* I could not follow the fate of this blastema, but in *Gallus* and *Larus* it was stated that a corresponding structure developed to form the well-known muscular process on the outside of the metacarpale 2.

(2) On the ulnar side of the hand the inturning of the head of the ulna has had the result that the ulnare and the 4th finger (HOLMGREN) no longer keep in line with the ulna (ulnar abduction) (fig. 23). The angle between ulna and ulnare in embryos 6 to 8 days old is occupied by a dense triangular mass of cells (fig. 23—26), which covers the distal end of the ulna and the ulnare on their outside and palmar side and extends distad to the rudiment of the 5th (HOLMGREN) carpale. In STEINER's pictures the distal tip of the triangular field in the 7½-day stage is developed as a "strahlförmige Anlage", die "ganz auf die palmare Seite des Basipodiums gerichtet ist." In my sections the triangular body is never "strahlförmig". The dense tissue closely covers the ulnare and is especially dense and thick on the palmar side of the ulnare. In this mass of tissue there develops a cartilage which fuses proximally to the ventral surface of the decreasing ulnare. According to STEINER (1922) this cartilage is the rudiment of metacarpale 5, which is said to have undergone a new flowering period. In my opinion this cartilage is nothing but a rather strongly developed pisiforme. This "finger" was later considered to be a pisiforme + metacarpale 5.

As the question of the 5th finger is of crucial importance, the answer to it must be supported by strong arguments. If we look for these arguments in STEINER's paper, we shall find that the only fact upon which he bases his interpretation of the pisiforme as the 5th finger is that its rudiment is "strahlförmig" and that it has shifted to the palmar side of the basipodium. This shifting is said to be a consequence of the so-called ulnar abduction, which has influenced the fingers of the wing of the birds. Such a shifting of the rudiment of the "5th" finger has really taken place ontogenetically. It develops actually on the lower side of the ulnare, where it keeps its position. There is thus not a single well established *fact* to substantiate STEINER's hypothesis regarding the "5th" finger.

In my view (and in that of other authors) the cartilage called by STEINER the 5th metacarpale (finger) is the rather strongly developed pisiforme. The reasons for this interpretation are:

(1) As birds are tetrapods they may be assumed to have a pisiforme.

(2) The pisiforme develops on a lower level than the ulnare, with which it fuses (if the ulnare is preserved). The ulnare + pisiforme is a hooklike bone, of which the ulnare portion is seen from the one side, the pisiforme from the opposite side (fig. 27).

(3) Into the posterior end of the bone (cartilage) the m.flexor carpi ulnaris is inserted, as is usual in Sauropsids and mammals.

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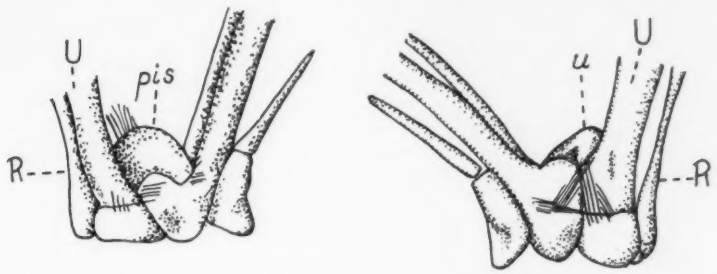

Fig. 27. *Anser anser*. Adult. Wrist seen from opposite sides.

(4) The anterior tip of the pisiforme is connected with the metacarpal bone by a strong ligamentous band. This ligament is the ligamentum piso-metacarpeum. A part of this ligament connects with the 5th (HOLMGREN) rudimentary finger and is the lig. piso-metacarpeum 5. This ligament is present in reptiles and mammals, where its ulnar part joins metacarpale 5, exactly as it does in birds.

(5) The insertion of the ligamentum piso-metacarpeum in the adult covers the rudiment of metacarpale 5, which in the adult *Anser brachyrhyncha* seems to remain as a small process on the palmar side of metacarpale 4. A big portion of the ligament is inserted in the big carpo-metacarpale.

(6) In a paper dated 1934 STEINER interprets the "5th metacarpale" (pisiforme) as metacarpale 5 + pisiforme, and in 1942 LUTZ considers it to be pisiforme + carpale 5 + metacarpale 5. As there is never any finger element that could be delimited from the pisiforme or seen to be inserted in the pisiforme, these opinions must be based on circumstances other than direct observation. Such circumstances are the following: "Ein Vergleich mit der embryonalen Vogelflügelanlage eines 8½-tägigen Embryos von *Anser anser* zeigt die grosse Ähnlichkeit, welche zwischen der ontogenetischen Entwickelung des Carpus der Vögel und jener der Krokodile besteht. In beiden treten die gleichen Elemente in einer beinahe gleichen Anordnung auf. Der einzige Unterschied besteht darin, dass sich im Vogelflügel das Centrale radiale distale mit dem Centrale radiale proximale (und dem Rudiment der Radiale) zum einzigen bleibenden, radialen Handwurzelknochen der Vogelhand verbindet, während am Aufbau des entsprechenden Elementes im Carpus der Crocodilier nur das Centrale radiale proximale, das Intermedium und das Rudiment des Radiale beteiligt sind." (STEINER 1934.)

This agreement is very superficial and consists largely in the fact that besides the pisiforme there are but two bones in the basal row of carpal bones. The radial one of them is, however, differently composed in the two groups (centralia 1 and 2 and intermedium in birds, intermedium and centrale 1 in croco-

diles). The system of centralia is also different, there being four distinct centralia in the crocodile, but three in birds (the 4th, the so-called centrale ulnare distale, being quite hypothetical, never having been seen in any bird). The disagreement between the radial and central parts of the carpus in crocodiles and birds solicits caution in using the former to explain the latter. STEINER continues: "Auch mit Bezug auf die Deutung des einzigen ulnaren, zweiten Carpalknochens des Vogelflügels, des sog. Pisiforme auctorum, gibt die embryonale Entwicklung der Krokodilenhand endgültigen Beschied. Ich schrieb 1922, dass der ulnare Handwurzelknochen der Vögel zum grössten Teil eine Neubildung sei, als deren Grundlage das Metacarpale V gedient habe". This important conclusion is based upon the following: "Die embryonale Krokodilhand zeigt aber dass die proximalen Elemente des fünften Fingerstrahles (Carpale 5 und Metacarpale V) in direkten Kontakt mit der Anlage eines typischen Pisiforme treten." In my paper of 1933 I have followed the development of the hand of the crocodile. My results were confirmed by STEINER (1934). From the photographs published there it is clearly seen that the 5th finger is inserted into the ulnare as is generally the case in tetrapods. The "direkte Kontakt" with the typical pisiforme is effected by the ligamentum piso-metacarpeum 5, which also joins carpalia 3—5 in *Alligator*. As the carpus grows longer during the development, the ligament grows longer as well. The only possible conclusion regarding the pisiforme that can be made from this evidence is that the pisiforme of birds is the same pisiforme as that in crocodiles, other reptiles and mammals (inclusive of man) and that there is no evidence that the pisiforme in birds includes any part of the 5th finger. The ligamentum piso-metacarpeum in crocodiles joins the 5th finger, therefore the finger which it joins in birds must also be the 5th, and not the 4th, as STEINER thought. Comparison with the crocodiles (and other reptiles and the mammals) thus affords strong evidence that the pisiforme of birds is merely a normal pisiforme and nothing else.

(7) Now, as it is perfectly clear that the ulnar finger rudiment in birds is that of the 5th finger, we must look for the 1st finger. The answer to this question has already been given, especially by NORSA (1895), MENNERT (1897), SIEGLBAUER (1911), PREIN (1914), HOLMGREN (1933 and in this paper) and MONTAGNA (1945). These authors have all found a finger rudiment basally to the first functional finger, between the carpale of this finger and centrale 2.

(8) As the pisiforme in birds is a normal pisiforme, its palmar position is quite normal. Therefore the "ulnar abduction" of the bird's hand cannot be responsible for the position.

I have treated the question of the pisiforme here at some, perhaps unnecessary, length because this is the only embryological or comparative anatomical point on which the hypothesis could possibly be based that the functional fingers of birds are the 1st, the 2nd and the 3rd.

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Larus fuscus (figs. 28—31, 34).

In my paper of 1933 I mistook the pisiforme for the ulnare and the ulnare for a "centrale 4".

In that paper I tried to interpret the central part of the hand according to the results obtained by STEINER in *Anser* and according to my own results in reptiles. This attempt led to the conclusion that the "radiale" of STEINER's description is no radiale but a centrale. This conclusion was confirmed by STEINER (1934). In 1933 I mentioned the existence of a true radiale, located between the radius and centrale. Simultaneously STEINER mentioned the same. The consequence of the recognition of the true radiale is that the "prepollex" of STEINER, which is inserted into the "centrale radiale distale" (STEINER) or centrale 2 (HOLMGREN), cannot be a prepollex but must represent a pollex, as I have interpreted it (1933). The research work I did in 1933 on the wing of *Larus canus* was somewhat superficial; I have therefore taken it up again on the basis of a more abundant material of *Larus fuscus*.

In an early 13 mm. stage the hand skeleton consists of ulna, ulnare, carpale 4 (+ 5) and the 4th and 5th fingers and a rudiment of the 3rd finger. The centrale complex and the carpal arch are almost confluent. The former consists of a broad band of blastematic tissue extending from the radial blastema in an ulnar direction, partly running to the ulnar column. This broad band (central blastema) is uniformly and rather densely cellulated. No special centres could be recognized in this field. Where it passes the head of the ulna it seems to be intimately connected with it, as there is no perichondrium separating it from the ulna. Radially it is continuous with the radial blastema. There are no differentiations either in the carpal arch or in the central blastema.

In a somewhat older 13 mm. stage the carpal arch is complete. It connects the bases of the four finger rudiments now developed. It contains two distinct thickenings, one for the 5th and 4th fingers and another for the 3rd. The central blastema contains two distinct centres at the tip of the radius and the ulna respectively. These two centres are fairly large so that they meet each other in the space between the ulna and the radius. The radial one of these centres is the rudiment of centrale 2. The ulnar centre contains the intermedium and centrale 3.

In the 15 and 16 mm. stages the ulna is distally turning in the medial direction (fig. 28). This inturning causes the "ulnar abduction" of the hand. It influences the centrale complex as it carries the condensation at the head of the ulna in the radial direction. Between this condensation and the radial blastema a zone has developed that foreshadows a new differentiation: the centrale 1 of the central complex. There are thus in the 15 and 16 mm. stages three fairly distinct centres located in a transversal row in the carpus: centrale 2, centrale 1,

Fig. 28. *Larus fuscus.* 15 mm. Hand.

Fig. 29. *Larus fuscus.* 18 mm. Hand.

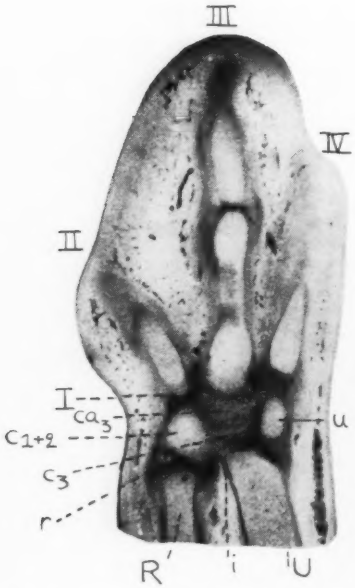

Fig. 30. *Larus fuscus.* 20 mm. Hand.

Fig. 31. *Larus fuscus.* 20 mm. Hand.

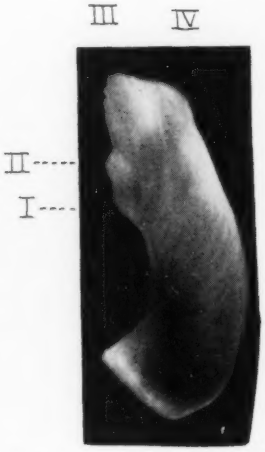

Fig. 32. *Struthio australis.* 14 days. Hand.

Fig. 33. *Struthio australis.* 14 days. Hand.

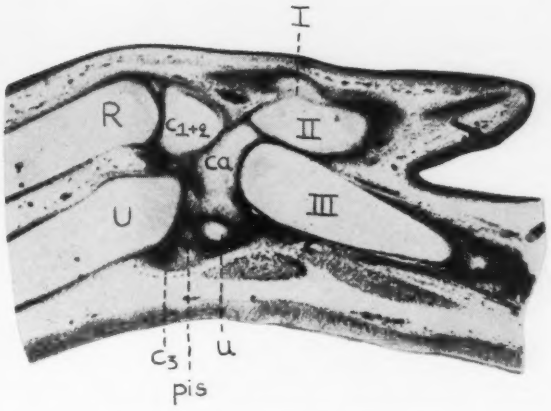

Fig. 34. *Larus fuscus.* 25 mm. Hand.

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and intermedium + centrale 3. A rudiment of the 1st finger is seen between metacarpale 2 and centrale 2.

In the 17 and 18 mm. stages (fig. 29) the first and second of the central condensations, counted from the radial side, are fairly well differentiated from one another. The third rudiment differentiates into a short intermedium part connected with the radial corner of the ulna, and an ulnar portion (centrale 3) extending in the ulnar direction towards the carpale of the 4th finger. A fairly well developed radiale (with prepollex) is wedged in between the radius and centrale 2. In this stage the central system very closely recalls the conditions in *Anser* (8½ days old) according to STEINER (1922). The rudiment of the 1st finger is the same as in the preceding stages.

In the 20 (fig. 30) *and 21 mm. stages* the different parts of the central system are better outlined than in the preceding stages. The inturning of the head of the ulna has carried the proximal part of the big blastema on its distal end somewhat more radiad, so that it now seems to connect the ulna with the second radial rudiment (centrale 1). The ulnar part of the rudiment on the head of the ulna, centrale 3, is as before. It is, however, somewhat flattened between the carpal arch and the head of the ulna. In the present embryos the 1st finger (prepollex according to STEINER) is so far developed that it causes a swelling on the radial border of the hand (fig. 31), proximal to the 2nd finger, as in the 14-day embryo of the ostrich (figs. 32, 33).

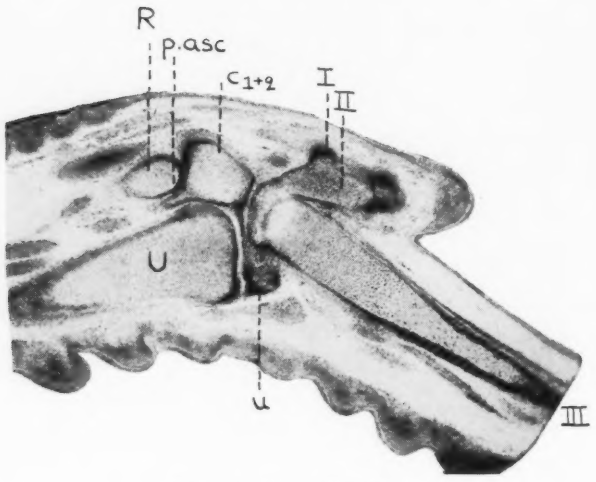

Fig. 35. *Pica pica.* Hand.

In the 23 and 25 mm. stages the centrale 1 rudiment has decreased and fused with the centrale 2. The intermedium rudiment is connected with the centrale 1. The centrale 3 is flattened and connected with the ulnare + pisiforme and with the radial border of the 4 (+ 5) carpale. These connections will later on be transformed into ligaments. In the 25 mm. stage the basal part of the 1st finger rudiment joins the metacarpale 2, forming the well-known muscular process on its radial side (fig. 34).

In an embryo ready for hatching the central complex consists of the same elements as in the 25 mm. stage, but they are differently developed. The two radial rudiments form together what has been called the "radiale". They correspond to the centralia 1 and 2 of my description and the centrale radiale proximale and distale according to STEINER (1934). The radial portion of the third rudiment is being transformed into a ligament. It is the intermedium. The ulnar portion is also tendinous, the tendon being inserted partly into the radial side of carpale 4 and partly into the (ulnare-)pisiforme. The latter tendon is the centrale 3 of my description and the centrale ulnare proximale according to STEINER. Ulnarwards the tendon widens[1] and connects with the ulnare + pisiforme complex. This widened part probably belongs to centrale 3.

Instead of the fingerlike process of the centrale 1 + 2, which in the 18-day *Struthio* embryo extends proximad between the *radius and the ulna*, there is in *Larus* a strong tendinous process, which proximally joins to the radius. Sometimes this process may chondrify. In *Pica pica* (fig. 35) the process is wholly cartilaginous and relatively more strongly developed than in *Struthio*. It is here-

[1] In *Pica pica* a rounded procartilage body corresponds to this widened part.

by stated that this process has nothing to do with the intermedium, which is simultaneously present. To some extent the process may correspond to the ascending process that is present in the foot.

During the development of the central complex the ulnar and radial borders have changed very little. On the ulnar side the ulnare and pisiforme have fused together, the ulnare-pisiforme forming a hooklike cartilage with the pisiforme directed frontad at almost right angles to the ulnare. The anterior tip of the pisiforme is continued by the ligamentum piso-metacarpeum, which ends at the rudimentary metacarpale 5 (4 according to STEINER).

The radial column consists of the radius and the radiale. The latter is quite rudimentary. It is a prochondrial, triangular body radially wedged in between the radius and centrale 2. This body becomes transformed into a ligament uniting centrale 2. with the head of the radius. There is no prepollex present.

Gallus domesticus.

In 1945 MONTAGNA published a paper on the development of the wing of the fowl. The results of this investigation are so different from those of other investigators (for instance PREIN) on the same subject and of those obtained from researches on other birds that it has been necessary to undertake a new investigation. I have, therefore, examined a large number of *Gallus* embryos (about 80). The chief result of MONTAGNA's study is that the functional fingers are the 2nd, 3rd and 4th. This conclusion is based upon the presence of a rudimentary first finger (distal carpale). He advanced the theory that there are three transversal rows of elements in the carpus: (1) five distal carpalia, (2) four centralia and (3) the basal row consisting of radiale, intermedium,ulnare, and pisiforme.

Any investigation of the morphology of the carpus must begin at a less advanced stage than that used by MONTAGNA. In his 5-day embryo all four fingers are developed, and well-defined condensations are apparently already visible in the carpus. If, however, we start with a very young 5-day stage[1], where but three fingers are developing, we shall find a fairly distinct cylindrical ulnare portion of the ulnar column between the ulna and the carpale rudiment. From the latter the blastematic carpal arch extends in a radial direction. This arch is very broad. Between the radial end of it and the radial blastema there is a narrow space poor in cells. In this stage, as in all the following ones, ulna and radius are of about the same length as, or the radius but a little shorter than, the ulna. The ulna is somewhat thicker than the radius.

In a 5-day stage, corresponding to fig. 2 in MONTAGNA's paper, there are three differently outlined condensations developing in the carpal arch. These corres-

[1] The development of the wings in different embryos during the same incubation period is not always the same. Sometimes it is retarded, sometimes more advanced.

pond to the centrale 4, centrale 2 and distal carpale 2 of MONTAGNA. How can that be?

The answer is: MONTAGNA has taken the somewhat independent rudiment of the epiphysis of the metacarpale 3 for a distal carpale. In the common fowl (and also in *Anser*) this rudiment is more strongly developed than in other birds and later joins on to the diaphysis.

As I have already stated, the ulnare arises early as a cylindrical blastema or procartilage. This rudiment is not visibly separated distally from the rudiment of carpale 4. The chondrification seems to begin distally, where carpale 4 and the distal part of the ulnare apparently chondrify together or in close contact with one another. When carpale 4 develops into the characteristic, thick and curved cartilage rod, it pushes the ulnare in the ulnar direction and proximad. Gradually the ulnare degenerates and seems ultimately to disappear, its cell nuclei becoming pycnotic. The possibility is, however, not excluded that a small remnant of the ulnare may be found on the dorsal surface of the posterior part of the pisiforme. The decrease of the ulnare may possibly be related in some way to the bending of the hand in the ulnar direction ("ulnar abduction"), which now becomes very pronounced as the head of the ulna simultaneously bends in the radial direction. Now the ulnare forms a small cartilage process on the dorsal side of the pisiforme. The pisiforme is just as MONTAGNA has described it, extending a process far distad to end below the metacarpale 4. In old stages this plate fuses on to the metacarpale distally. Metacarpale 4 has a long radial process extending below the carpus to its mid-line, where it reaches the tendon of the big flexor of the fingers. The distal end of the pisiforme is joined by a ligament (ligamentum piso-metacarpeum) with metacarpale 5 (as is also the case in *Struthio, Anser* and *Larus*).

The ulnare, as described in this paper, was considered by me in 1933 to be a centrale 4. This interpretation was due to my mistaking the pisiforme for the ulnare, a mistake caused by the ligamentous connection (ligamentum piso-metacarpeum) between the pisiforme and the 5th finger. At the time I considered the ligament to be a remnant of a closer connection (articulation) between the "ulnare" and the 5th finger.

Distally to the radius there lies a rudimentary radiale wedged in between the radius and a big cartilage, which I (1933) interpreted as consisting of two components: the centralia 1 and 2 according to my terminology, the radiale and centrale radiale according to that of STEINER (1922). Later (1934) STEINER changed his opinion, considering the two components to be a centrale radiale proximale and a centrale radiale distale. LUTZ (1942) followed STEINER'S interpretation. Now, MONTAGNA does not consider the cartilage in question to be a compound structure and calls it simply centrale 1. The observed conditions in *Gallus* actually do not allow of any other interpretation, but in *Larus* it is obvious that the corresponding cartilage is a compound structure and in also

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Struthio it is almost certainly so. According to STEINER (1934) the same is the case in *Anser*, and according to LUTZ (1942) in *Dromiceius* also. We must, therefore, assume that this is likewise the case in *Gallus*, though the duplexity does not show up clearly during the embryonic development.

At the distal end of the ulna the outline of this cartilage is uneven because there is no perichondrium present. Therefore the cartilage is without a boundary line running continuous with the blastema distal to its head. This head is in 6- and 7-day embryos rather strongly inturned so that it more or less approaches centrale 1 + 2. The blastema at its head is partly directed toward centrale 1 + 2, partly directed right distad, forming a pad located between the ulna and the distal carpale 3. The first-named blastema constitutes the rudiment of the intermedium, and the other results in the formation of centrale 3. The intermedium does not chondrify in *Gallus* but is transformed into a short ligament joining the ulna with centrale 1. Centrale 3 becomes flattened out and ligamentous. Towards the ulnar border this ligament in the 9-day chick is inserted partly in the reduced ulnare and the pisiforme, partly in carpale 4 (compare *Larus*).

MONTAGNA	HOLMGREN
functional fingers II—IV	functional fingers II—IV
distal carpale 1	1st digit
„ „ 2	carpale 2
„ „ 3	proximal epiphysis of metacarpale 3
„ „ 4	carpale 4
„ „ 5	5th digit
centrale 1	centrale 1 + 2
„ 2	carpale 3
„ 3	centrale 3
„ 4	ulnare (in part)
ulnare + pisiforme	ulnare + pisiforme
radiale (rudiment)	radiale (rudiment)

b. Central parts of the tarsus

Struthio australis.

As stated above (pp. 00—00) the central part of the tarsus is already developing in *the 9-day stage*. The foot there consists of the tarsal arch with toes 5, 4 and 3. The 2nd toe, when developing, will join on to the fibular end of the tarsal arch. Proximal to the tarsal arch a transversal band of dense mesenchyme, running over the space between the tibial border and the rudiment of the fibulare, represents the 1st toe (radially) and the central complex. The toe rudiment is still only a heap of mesenchyme inserted in the radial end of the central band. This

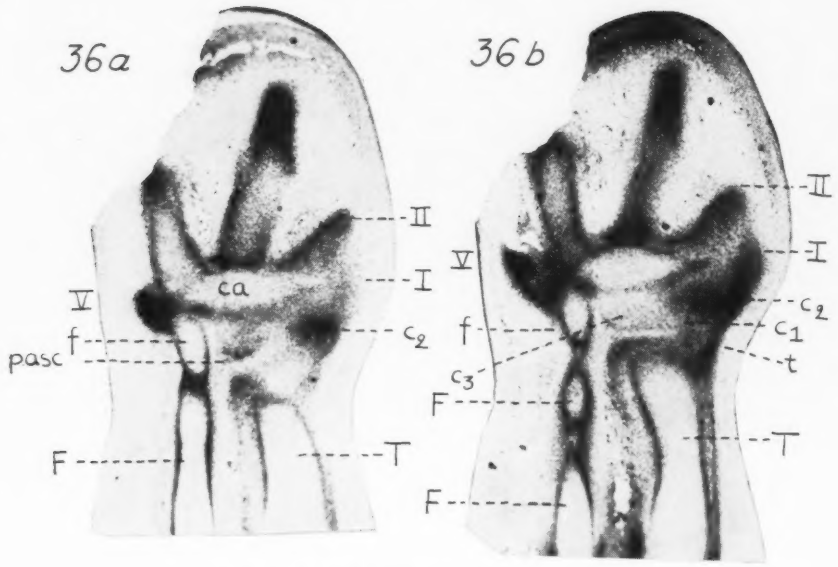

Fig. 36. *Struthio australis.* 10 days. Foot.

band already consists of three different parts: (1) a thick tibial mesenchymatic body — centrale 2, (2) a central, fairly thick body — centrale 1, and (3) a narrow, thinly cellulated portion — centrale 3, connected with the fibulare. When following the series of horizontal sections in a dorsal direction we see that the superficial part of the centrale 1 portion becomes free from the rest of the transversal band, which thus has a dorsal (rounded) process. This process is the rudiment of the so-called "ascending process" of the tibiale or intermedium. It is connected with the tibial corner of the fibula by means of a thin, rather diffuse strand of mesenchyme. As this strand is the only connection between the ulna and the central system, it probably represents a very rudimentary intermedium. It is possible, however, that the strand is the rudiment of a tendon, which has once connected the true intermedium with the ulna. In that case the intermedium has become attached to the dorsal surface of centrale 1, there forming the "ascending process". The question of the "ascending process" will be discussed separately.

The 10-day embryos (fig. 36, a and b) have five developed toes. The 1st however, is very small and mesenchymatic, whereas the others are prochondrial. The tarsal arch is complete from the 2nd to the 5th toe. The various tarsalia are not differentiated from one another, the entire arch being a cartilaginous disc with which the toes articulate. The different parts of the central complex together form a single large cartilage disc. The processus ascendens is fairly well developed. The intermedium, easily recognizable in the 9-day embryo, is repre-

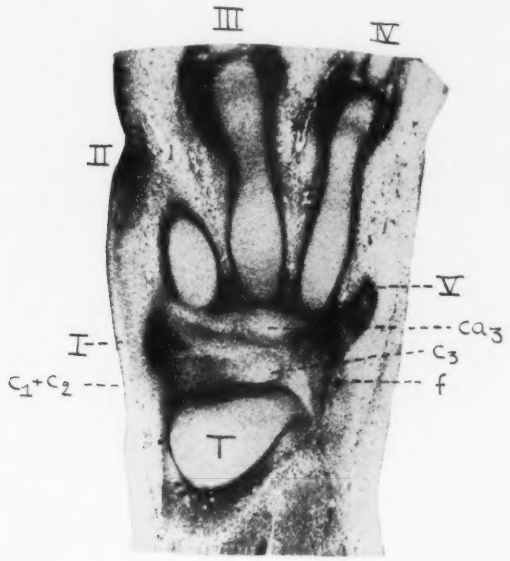

Fig. 37. *Struthio australis.* 11 days. Foot.

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sented in the 10-day embryo by a strand of mesenchyme extending from the distal fibular corner of centrale 1 to the distal end of the fibulare. The tibiale of this stage is prochondrial, covering the tibial distal corner of the tibia. The tibiale is apparently confluent with centrale 2. The 1st toe articulates partly with centrale 2 and partly with the tarsal arch.

The tarsal arch of *11- and 12-day embryos* is a single lens-shaped cartilage (fig. 37). Where the 3rd toe articulates with it, it has a well outlined lens-shaped thickening-tarsale. 3. For the other toes there are no differentiated tarsalia, except possibly for the 5th, which seems, at a certain stage of development, to have a small tarsal of its own, connecting with the main tarsal arch. The well-developed, strongly chondrified fibulare has on its lower side a longitudinal ridge, which is connected distally with the 5th toe. This ridge apparently corresponds to the "pisiforme" described by LUTZ (1942) in the emu (*Dromiceius*) and with the fibulare of my description of *Larus* (1933). Between this ridge and the centrale 1 part of the centrale is a well-delimited space, poor in cells (fig. 40). The fibulare and the "pisiforme" are located just outside this space. From the centrale the chondrification proceeds in a fibular direction, so that ultimately the fibulare and the "pisiforme" become incorporated with the central disc. The connection between centrale 1 and the fibulare + "pisiforme" represents centrale 3.

The "ascending process" has now widened its basis, so that it issues from the posterior and dorsal margin of the entire central complex (+ the radiale). On

the fibular side it is also connected by a commissure with the fibulare and the fibula. The original connection with the "intermedium" is no longer visible.

The 13 and 15—18 days old embryos do not differ from the 11—12 days old specimens in any fundamental way, but the chondrification is more advanced in all parts. The fusion of the "pisiforme" with centrale 1 by means of centrale 3 is nearly complete in the 15-day stage, and in the 18-day embryo adult conditions seem already to be established.

Anser anser, Larus fuscus, and Gallus domesticus.

The development of the foot in *Anser, Larus* and *Gallus* follows the same lines as that of *Struthio,* and there are scarcely any differences of importance. In *Larus,* however, where a "processus ascendens" is well developed, this structure is similar to that in *Struthio,* whereas it is absent in *Gallus.* Nevertheless, the centrale 1 of *Gallus* has a weak dorsoproximal projection, which may represent an ascending process.

5. FIRST FINGER IN BIRDS

In the preceding pages evidence has been given for the functional fingers of the bird's hand being the 2nd, 3rd and 4th. The 1st and the 5th fingers are thus missing. It is well known that the 5th finger is fairly well developed in the embryo, but the existence of the 1st finger has been denied. I shall therefore here take up first of all in some detail the question of the 1st finger.

In the foot rudiment of a 5 days 4 hours chick the 2nd toe rudiment differs in shape from the others, being conical. In its external surface is inserted the less developed rudiment of the 1st toe. In the hand of a 5 days 10 hours chick the 2nd finger is also conical, and on its external side it carries a small, rather diffuse but nevertheless well discernible rudiment of the 1st finger. In the hand of a 15 mm. embryo of *Larus fuscus* the 1st finger is developed identically. In the foot of a 6-day chick the 1st toe is normally developed with the "centrale 2" but adheres medially to the base of the 2nd toe. In the corresponding location in the hand of a 6 days 8 hours chick a weak rudiment of the 1st finger adheres to the base of the 2nd finger.

In the hand of a 11—12 days old ostrich the rudiment of the 1st finger is fairly distinct in the outside of "centrale 2". In these stages a "prepollex" is easily discernible at the external corner of the radius. Thus a 1st finger and a prepollex are present simultaneously. Consequently the identification of the "1st finger" with the prepollex (STEINER) cannot be correct.

Thus, wherever in the embryos the rudiment of the 1st finger appears, it adheres to the base of the 2nd finger. At that place we now find in the 20 and 23 mm. *Larus* embryos a pad of prochondrial tissue, into which the m. flexor metacarpi radialis is inserted. This pad is the rudiment of the 1st finger. Fol-

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lowing its extension, we find it to be a cylindrical rod extending towards the dorsal surface of the hand, where it pushes out the skin and forms a small rudimentary finger. In *Struthio* a similar "finger" is also present, but there I could not follow its development fully. MEHNERT has described the 1st finger in *Struthio* as a narrow process located on the outside of the metacarpale 2. In a corresponding location there was in a 13-day ostrich a small rudiment that may probably be that of this process.

The last step in the development of the 1st finger is taken, for instance, in a 25 mm. *Larus* embryo, where there is a small cartilage on the outside of metacarpale 2. Into this cartilage the m. flexor metacarpi radialis is inserted, and this cartilage must consequently represent the 1st finger. This cartilage is fused on to metacarpale 2 and there forms the well-known muscular process of this metacarpale. This process is thus the 1st finger, which has survived by changing its function.

6. THE ULNAR ABDUCTION OF THE HAND IN BIRDS

"3. Die ulnare Abduction des Antepodiums führt im Carpus der Vögel zur aufeinanderfolgenden und vollständigen Reduktion des i[1], des cu und des u. Das cr verschmilzt mit dem r zum radialen, bleibenden Handwurzelknochen der Vögel.

4. Die ulnare Abduktion bewirkt die sukzessive und von der ulnaren Seite her eintretende Reduktion der Fingerstrahlen, so dass zuerst der V., dann auch der IV. Finger vollständig unterdrückt werden. Heute ist auch der III. schwächer als der II. entwickelt, und die Reduktion der Phalangen setzt sich sogar bis auf die erste Phalange des II. Fingers fort. Der proximalste Teil des V. Fingerstrahles hat jedoch eine sekundär wieder einsetzende, stärkere Ausbildung erfahren. Er bildet heute den zweiten, bleibenden, ulnaren Handwurzelknochen der Vögel und stellt somit weder ein u, noch ein pi dar, noch ist er mit irgendeinem anderen Elemente des Carpus verschmolzen. Zum grössten Teil ist er eine Neubildung, doch hat das mcV als seine Grundlage gedient." (STEINER, 1922, p. 329.)

Further it is stated that the ulnar abduction causes the ventral dislocation of the reducing elements of the bird's wing.

Our conception of these effects of the ulnar abduction is, however, influenced by the interpretation of the functional fingers as the 2nd, 3rd and 4th fingers instead of the 1st, 2nd and 3rd.

The abduction appears much later than is supposed by STEINER, in a stage of development when the development of the different parts of the hand is already fairly advanced. The first sign of an ulnar abduction seems to me to be when

[1] i = intermedium; cu = centrale ulnare; u = ulnare; cr = centrale radiale; r = radiale; pi = pisiforme; mcV = metacarpale V.

the head of the ulna bends radiad so that the ulnar support of the hand shifts from the ulnar border cartilage (ulnare) to the intermedium-centrale complex.

Through this shifting of the point of support of the hand the posterior part of the ulnare is brought outside the head of the ulna. As by the shifting its supporting quality has decreased, it is conceivable that it has become reduced or fused to the pisiforme. The carpus is thus now chiefly supported by the two centralia on the radial side and the pisiforme on the ulnar side. This accounts for the strong development of the pisiforme, which, lying as it does palmar to the rest of the carpus in spite of the ulnar abduction, could develop freely in the medial as well as in the frontal direction. The inbending of the ulna apparently caused the flattening of the centrale 3 of the central complex. This abduction could not have anything to do with the reduction of the 5th finger[1].

It is obvious that the inturning of the head of the ulna causes the abduction that generally occurs in carinate birds. In the embryos of *Struthio* the ulna does not turn mediad, but the ulna and radius are parallel or even slightly divergent distad. In these embryos there is no ulnar abduction until the 18-day stage, at which a considerable ulnar abduction may be said to occur. But already in the 19-day stage as well as in the 21-day stage and in half-grown embryos the abduction is radial, later to become ulnar again. In *Dromiceius* the ulna and radius diverge distad, and there is no trace of any inturning of the ulna either in the 15- or in the 20-day stages. In *Casuarius* the abduction, if any, is very inconsiderable in the adult, but in *Rhea* it is more pronounced than in *Struthio*.

From the related facts only one observation can be made: The ulnar abduction of the hand begins much earlier in the carinate birds than in ostriches. The abduction in the carinates begins with the inturning of the head of the ulna, while in the ostriches no marked inturning occurs.

In ostriches the head of the ulna and also that of the radius grow considerably in volume, so that the two heads come closely together. As, however, the head of the ulna is stronger than that of the radius, the centre of gravity of the hand comes to rest primarily on the ulna. In the Carinates the same effect is attained in another way — by the inturning of the head of the ulna.

If we assume that the inturning of the ulna and extreme ulnar abduction are adaptations for flying in the carinate birds, we must ask what kind of adaptation is the non-appearance of the inturning in ostriches. Does that perhaps signify that these birds have lost their property of flying secondarily or that they have never been flying birds? Both theories have their advocates.

[1] The ulnar abduction referred to here is not the same as the early abduction of which STEINER speaks. The ulnar abduction of STEINER rests upon the misinterpretation of the 5th finger as being the 4th. As the 5th finger is always abduced in an ulnar direction, this abduction of the "4th finger" can be disregarded.

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STUDIES ON THE PHYLOGENY OF BIRDS

7. HOMOLOGIES (HOMODYNAMICS) BETWEEN HAND AND FOOT IN BIRDS

In 1933 I tried to draw a comparison between the hand and the foot in *Larus*. This comparison was in some points unsatisfactory as far as regards the central system. The preceding investigation has resulted in some partly new points of view concerning the homologization.

About the comparison of the fingers and toes as well as of the carpalia and tarsalia there can be no fundamental difference of opinion, but the central system has been interpreted in different ways. Most of these differences have already been dealt with in preceding pages, so that I can restrict myself to the results of my investigation.

The big cartilage lying in the wing distal to the head of the radius is composed of the centrale 2 (radially) and the centrale 1 (medially). On the centrale 1 portion there is sometimes (*Struthio, Pica*) a process running proximad and entering between the ulna and radius. This process I have provisionally called processus ascendens. In *Struthio,* where a chondrified intermedium is present, it fuses with the dorsal side of this process. In *Pica,* where there is no chondrified intermedium, the process is single. From the centrale 1 the centrale 3 extends to the ulnare and pisiforme. The 1st finger is located on the centrale 2, the 5th on the ulnare. The latter, however, is joined to the pisiforme by a ligament. A rudimentary radiale is present between the head of the radius and the centrale 2.

In the foot a big cartilage plate covers the head of the tibia. This cartilage is composed of the centralia 1—3. On the centrale 1 portion there adheres a component, which in early stages of development is connected with the radial corner of the fibula. This component must therefore be the intermedium. This part of the centrale 1 together with the "intermedium" sends out a dorsal process in the space between the tibia and the fibula. This process is the so-called ascending process of the tibiale. As the embryo grows older, the ascending process widens its base, so that it involves the entire centrale and tibiale and extends towards the fibulare. This process corresponds in part to the processus (ascendens) of the hand (in *Struthio*). The centrale 2 is joined with the fibulare (and "pisiforme") by a short centrale 3. The first toe is located on the centrale 2, the 5th on the fibulare. A ligament also connects the 5th toe with the "pisiforme" part of the fibulare. A rudimentary tibiale is present between the head of the tibia and the centrale 2. The 3rd toe and the 3rd finger are generally the longest ones.

Comparing the two last summaries, we thus see that the hand and the foot of birds are built after the same pattern, in spite of their very different external appearance and function.

8. SOME PHYLOGENETICAL CONSEQUENCES

All attempts to trace the phylogenetical developments of the hand in birds seem to have started from the interpretation of the functional fingers as being the 1st, 2nd and 3rd fingers of the pentadactyle hand. Now, however, knowing that this interpretation is incorrect and that the fingers are the 2nd, 3rd, and 4th, we must view this development in a different way from before.

The first conclusion we must draw from the preceding statement is that the fingers of the wing of *Archaeornis* and *Archaeopteryx* are the 2nd, 3rd, and 4th fingers, and that the ancestors of those birds must therefore have reduced the 1st and the 5th fingers and not the 4th and 5th as is generally believed. Thus such extinct reptiles as are reducing or have reduced the 4th and 5th fingers must be excluded from the nearer avian ancestry. Excluded are therefore such theropod types as *Anchisaurus, Gresslyosaurus, Ceratosaurus, Allosaurus, Anthrodemus, Gorgosaurus,* and Ornitischians such as *Hypsilophodon.*

In his "Grundzüge der Palaeobiologie der Wirbeltiere" (1912) ABEL has treated the problem of the development of the avian hand. He derives it from the hand of such dinosaurian types as *Gresslyosaurus, Allosaurus,* and *Plateosaurus,* in which the 4th and 5th fingers are reducing or already reduced. To these he adds the Compsognathids. His opinion about the three fingers of the *Archaeopteryx* hand is that they are "trotz der scheinbar abweichenden embryologischen Resultate bei den lebenden Vögeln als der I-III Finger zu betrachten." ABEL has arrived at this standpoint because of the phalangeal formula of the *Archaeopteryx* hand and that of the diapsid reptiles. BROOM (1906) also considered the phalangeal formula decisive for the determination of the fingers in *Archaeopteryx.*

When some authors point out that the phalangeal formula of *Archaeopteryx* corresponds to that of the three first fingers in diapsid reptiles and conclude from this correspondence that the functional fingers of the recent birds are these three fingers, they have tackled the problem in the wrong way. They have forgotten that it is as yet impossible to analyse the *Archaeopteryx* hand without a deep knowledge of the hand of recent birds. In the hand of *Archaeopteryx,* as we know it, two fingers are missing. The fossils give no evidence whatsoever as to where the two missing fingers were situated. But in recent birds — in which an analysis can be made — the 1st and the 5th fingers are missing. Therefore the unavoidable conclusion is that this was the case in *Archaeopteryx* as well, and that thus *Archaeopteryx* and its predecessors had already reduced their phalangeal numbers. Moreover, in the case of the birds it is quite natural that the phalangeal numbers of the hand should differ from those of the foot. The adaptation-for-flight of the hand that had already begun in the ancestors of *Archaeopteryx* must have influenced the phalangeal numbers.

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In spite of the reluctance one must feel to assume that there has already been a reduction of the phalangeal numbers in *Archaeopteryx*, one cannot get away from the facts which have resulted from the embryological investigation. A comparison — made on the basis of these facts — is given in the following table:

Phalangeal formulae of the hand.

Diapsids, generally	2,3,4,5,2.	
Crocodiles	2,3,4,4,3.	
Cameleonts	2,3,4,4,3.	
Molochus	2,2,3,3,2.	
Chelonia	2,3,3,4,3,	to 2,2,2,2,1.
Procompsognathus	2,2,3,3-4,2[1]	
Archaeopteryx and Compsognathids	–,2,3,4,–	
Struthio	-,2,3,2,–	
„ (WRAY 1887)	–,2,3,3,–	or –,2,3,4,–[2]
Carinate birds	–,1,2,1,–	

These figures clearly show that a reduction of the number of the phalanges in the hand is already a rather common phenomen among the reptiles and that such a reduction has taken place among recent birds. There is no reason why the reduction should have been restricted to recent birds only and not have begun already earlier than in the Saururae (or Compsognathids). In the feet there is generally no corresponding reduction. As the hand and foot formula may once have been the same, the reduction of the phalanges of the hand probably occurred before the *Archaeopteryx* stage.

From the point of view of the phalangeal reduction we now have to look for acceptable ancestors of *Archaeopteryx* and the other birds. When looking for ancestors to the birds different authors have approached some Coelurosaurs such as *Ornitholestes, Compsognathus* and *Struthiomimus,* the 3-fingered hands of which were said to have arisen through reduction of the 4th and 5th fingers. It was simply assumed that the 3-fingered hand of the Compsognathids arose in the same way as the functionally 3-fingered hand of *Anchisaurus, Gresslyosaurus, Ceratosaurus* or *Anthrodemus,* through reduction of the two ulnar fingers. If, however, one compares the hand of *Archaeornis* with, for instance, that of *Ornitholestes,* as has been done many times, one is struck by the very large measure of agreement: the three metacarpalia lie close together, parallel

[1] This formula is not quite certain, as it is made up from a not fully preserved hand described by von HUENE.

[2] WRAY (1887) stated that the last phalange of the "3rd" (4th) finger is a compound structure consisting of at least two phalanges fused on to one another. He suggests in addition that the apical one of these two phalanges may also contain two elements. My observations suggest that WRAY is right, but I cannot positively state that this is the case.

with each other, the radial metacarpale is short in both cases and possesses an external concavity[1].

The ulnar finger is much weaker than the others. But the most striking agreement is the radial abduction of the radial finger in the Compsognathids as well as in *Archaeopteryx*. If this agreement is more than a coincidence, we must conclude that the radial finger in the Compsognathids is the 2nd finger, just as in *Archaeopteryx*. The Compsognathids thus probably have already lost their 1st finger or that finger has been cartilaginous and therefore could not be preserved. The same was apparently the case with the 5th finger, of which, however, the metacarpale may be present.

Considering the very close general agreement between the Compsognathids and the Jurassic birds, the conclusion seems well justified that there was some genetic connection between them. That is a conclusion that has been drawn many times (see HEILMANN 1926).

Among other points of agreement between Saurischians and birds I may here only mention the presence of a processus ascendens in both groups. This process is said to be absent in the Ornithischians.

There are, of course, disagreements as well, and such are present in most characters, but they do not seem to be too wide to permit of their being easily overbridged. HEILMANN has presented a useful comparison concerning a number of characters. The most important disagreement seems to me to occur in the development of the pelvis in the two groups.

There are two main types of dinosaurian pelvis, viz. the saurischian and the ornithischian ones. The former is 3-rayed, the latter 4-rayed. In the Saurischians the pubis and ischium are strongly divergent downwards, in the Ornithischians they are parallel with each other, both directed backwards. The processus pectineus in the Saurischians, if present, is weakly developed, in the Ornithischians it is very long and strong, directed frontad. In *Archaeopteryx* and *Archaeornis* the pelvis is 3-rayed, with pubis and ischium directed caudad but remarkably divergent. A pubic symphysis is present, the processus pectineus is absent. In *Struthio* the pubis and ischium are directed caudad, nearly parallel but well separated from each other except caudally, where they are joined. On the pubic cartilage a rather strong processus pectineus is present. At about the middle of the pubis a thin cartilaginous process is present on its lower side. A pubic symphysis is present. In the other struthious birds the pelvis differs from that of the ostrich differently in the different genera. In *Rhea* and *Casuarius* the pubis and ischium are joined at their posterior tips and the ischium is, moreover, joined to the ilium. In *Dromaeus* the conditions are the same, but the junctions are cartilaginous. In *Apteryx* the pubis, ischium and ileum are separate. In the Carinates the pubis is generally a slender rod, whereas the ischium is a plate or

[1] It seems very probable that at one time this concavity contained a rudimentary 1st finger.

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rod, generally connected with ilium and with pubis. Both bones are directed caudad. There is no symphysis. The pectineal process belongs to the ilium.

Among the birds there are thus at least three types of pelvis: the *Archaeopteryx* type, the struthious type and the Carinate type. The question is now: to which of the saurian types, the saurischian or the ornithischian, are the avian types most closely akin? Both pelvis are rather unlike the avian one, but the 3-rayed pelvis of a Saurischian undoubtedly disagrees more with the avian pelvis than does the 4-rayed pelvis of the Ornithischians. On the other hand, the general characters of an *Ornitholestes* agree far more closely with those of *Archaeornis* or *Archaeopteryx* than any Ornithischian does. Therefore it seems necessary to try to make an embryological analysis of the avian pelvis in order to find a way out of this contradiction.

It is a well known fact that ontogenetically the pelvis of modern birds begins its development as a 3-rayed structure not very unlike that of a Compsognathid. The pubis of the embryonic pelvis of a Carinate, however, soon bends caudad and becomes parallel with the ischium. In the same way, fundamentally, the Carinate pelvis is developed. There are no symphysis in that pelvis. It differs very much from that of the Saururae (*Archaeornis* and *Archaeopteryx*), which have a long pubis with a long symphysis and a short ischium without a symphysis. The pubis and ischium diverge considerably caudad. The sauruan pelvis agrees more closely with that of *Struthio* but less with that of the carinate birds. Accordingly it must be of some interest to try to make an embryological analysis of the pelvis of the ostrich. Maybe it could give some information about the relations between the saurischian pelvis and that of birds. I have therefore investigated the development of the pelvis in some ostrich embryos.

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9. DEVELOPMENT OF PELVIS IN STRUTHIO AUSTRALIS

The pelvis of one 10-day and one 11-day stage of Struthio have been described by Broom (1906). According to him the acetabulum is completely closed in the 10-day stage. The pectineal process is large. It appears to be formed by the pubis rather than by the ilium. The pubis is a slender cartilaginous rod that curves slightly backwards. Its lower end passes into a broad sheet of procartilage, which connects it with the ischium. Near the lower end of the pubis a condensation of procartilage represents the "prepubis". There is no symphysis of the pubis or the ischia. In the 11-day stage the pelvis is more or less as in the adult. "The conditions of the pelvis are particularly interesting as showing that the avian type is not derived from one with the pubis and ischium apart, as in the carnivorous Dinosaurs, but rather a modification of such a type as is seen in the Pterosaurs. It further seems to show that originally the pubis and the ischium were directed downwards and that the backward direction is a late modification." The authors (Johnson 1883, Lebedinsky 1913), who have described the develop-

ment of the pelvis in carinate birds, agree that the pubis and ischium grow out in ventral, somewhat diverging directions. Then the pubis bends caudad and runs parallel with the ischium. This description also holds true concerning *Struthio,* but there are some important points to be added to that simple description.

The pelvis of a 10-day embryo is 3-rayed, the chondrification is beginning separately in the ilium, pubis and ischium. The three cartilages together form the acetabulum, which is wide open. The ilium is a considerable cartilage situated along the vertebral column. The pubis is developed vertical to the ilium. On the upper end of the pubis a large processus pectinealis extends frontad. From this part the pubis bends slightly in the caudal direction, then slightly frontad and downward. On the frontal side, just where the cartilage bends down, an obtuse procartilaginous or blastemic process is situated, with which the rudiments of certain abdominal muscles (mm. rectus and obliquus abdominis externus) connect. That portion of the pubis which is bent caudad is short and ends in an undifferentiated medial field of connective tissue or blastema, at least partially representing a symphysis. The rudiment of the ischium is a somewhat conical rod forming an angle of about 45° with the pubis. It is thus directed obliquely caudad. Its distal end appears to abut on the medial field mentioned above.

As in the preceding embryo, the three components of *the pelvis of the 11-day embryo* are independent or nearly independent of one another. The processus pectinealis of the pubis is very strong. The angle between the pubis and the ischium has become considerably smaller. The lower part of the pubis is bent caudad, and just before the bend it has a prochondrial frontal process, which serves for the insertion of the mm. rectus and obliquus abdominis. Caudally the lower part of the pubis ends in a very large prochondrial mass: the rudiment of the future symphysis pubis and the cartilaginous, later ossifying, ventral connection between the pubis and the ischium. The ischium is remarkable. It is about as long as the vertical part of the pubis. Along its frontal border runs a prochondrial band ("pre-ischium"), of which the broadened ventral part runs into the big prochondrial mass mentioned above. The anterior part of this mass later forms the symphysis pubis, the posterior part forms the connection between the pubis and the ischium.

In a 13-day embryo the caudad bent part of the pubis has grown longer than before and is connected with the frontal part of the symphysial mass. This frontal part forms the well-known hook at the symphysis of *Struthio.* The "pre-ischium" is now divided into a short dorsal portion, the interobturator process, developing into the insertion crest of the ischio-femoralis muscle, and a big ventral mass forming the ramus inferior ossis ischii or the skeletal lamella caudally joining the pubis with the ischium. From the anterior process of the

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pubis, into which the abdominal muscles are inserted, there issues a broad triangular collagen lamella or aponeurosis.

10. COMPARISONS IN REGARD TO THE PELVIS

The only type of pelvis with which the 10-day stage of the pelvis of *Struthio* can as yet be successfully compared is that of a saurischian Dinosaur, for instance, that of a Compsognathid. In those reptiles, which have a 3-rayed pelvis, the distal end of the pubis has a horizontal "foot" or "peduncle". This foot has an anterior short process, "the toe", and a posterior longer process, "the heel". The latter formed a symphysis, whereas the former probably served for the insertion of abdominal muscles and perhaps the system of abdominal ribs probably developed in connection with the m. rectus abdominis. In the 10-day ostrich embryo the pubis likewise ends ventrally with a "foot", which, however, is directed almost vertically. Into the "toe" of this "foot" abdominal muscles are inserted, and the "heel" abuts on to an undifferentiated mesenchyme mass. The pubis of the ostrich differs from that of the Compsognathids as it has a well-defined pectineal process, but in other Saurischians there is a process that might correspond to a pectineal process, for instance, in *Tyrannosaurus*.

In the 11- and 13-day embryos the "heel" of the pubis has developed into a strong caudal portion of the pubis, which is caudally connected with it by means of a symphysis with the corresponding part of the pubis on the other side. The strong growth of the pubis has caused it to exceed the ischium in length. The development of a "pre-ischium" in the 11-day stage has in the 13-day stage resulted partly in the formation of the cartilage plate that joins the ischium with the pubis, partly in the appearance of the obturator process common to Dinosaurs and birds.

Pelvis of Saururae

Thus the ontogeny of the ostrich pelvis makes it possible or at least not impossible to explain the avian pelvis as being derived from a 3-radiate dinosaurian pelvis. As the pelvis of *Archaeopteryx* and *Archaeornis* probably belong to the avian type, akin to the ostrich type, it may also be explained as having arisen from a saurischian pelvis type. Here follows an attempt to explain the *Archaeopteryx* pelvis from the evidence gained from the embryological development of the pelvis of the ostrich.

The avian character of the sauruan pelvis is emphasized by the backward direction of pubis and ischium. There is a pubic symphysis, but the two ischia are rather strongly divergent. The "symphysis" is formed by the lower halves of the two pubes meeting each other without fusion in the midline. Behind the tip of the pubis there is an oval plate or body of calcite, which probably represents original cartilage (PETRONIEVICS & WOODWARD 1917). In the Berlin specimen

the distal body seems to be ossified and compressed from the sides. The ischia are much shorter than the pubes and bifurcate at their tips. A pubis such as that of the Saururae is only met with in some Coelurosaurs. In *Coelurus* the symphysis is much longer than in the Jurassic birds. This symphysis ends with a strongly developed "foot", which is bent dorsad, so that it is not seen from the front. The appendix of the sauruan pubis may represent the "heel" of this "foot". Assuming that our embryological analysis of the pelvis in the ostrich is applicable to the pelvis of the Saururae, the appendix of the pelvis of the latter must correspond to the "heel" in the early stages of the ostrich.

In the ostrich the ischium is composed of an anterior part: the "pre-ischium", and a posterior part: the ischium. The ischium here can thus be described as bifurcate. Applying this knowledge to the sauruan ischium it seems fairly probable that the two terminal branches have the same significance as those in the ostrich. In the ostrich the "pre-ischium" joins the pubis somewhere in the middle of its "heel" portion. In the Saururae, where the "heel" portion is very short, the "pre-ischium" may have been connected with it by a ligament or a fascia, developed from the "pre-ischium". How this fascia was placed cannot be determined with any certainty, but comparing it with that of the 13-day *Struthio* embryo, in which it joins the pubis just in front of the short apical symphysis, we may conclude that it has run between the ventral process of the ischium and the proximal part of the pubic appendix ("heel") in the Saururae. It may be mentioned that the pubis and the ischium diverge caudad and that the ischium is by no means parallel with the lower border of the ilium. The ventral pelvic bones thus hold a position that could almost be described as intermediate between that in the Saurischians and in struthious birds.

Pelvis of Saurischians

If, as we have seen, it is possible to explain the ostrichian pelvis as being derived from a saurischian one, it must also be possible to obtain some imformation about the saurischian pelvis from the conditions in the ostriches. In the Saurischians there is a pubis as well as an ischium symphysis. If the conditions are like those in the ostrich, the pubis and ischium were connected with a ligament or cartilage. This ligament should thus correspond to the ligamentum medianum as it is developed in recent reptiles, and it should be developed from the "pre-ischium." At its upper end the ischium carries the obturator process in ostriches as well as in Dinosaurs. The "pre-ischium" must, at least in the embryo, have been located along the anterior margin of the ischium.

Pelvis of Ornithischians

The interpretation of the pelvis of birds depends largely on the interpretation of the pectineal process. Generally it is considered that this process of the

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bird's pelvis belongs to the ilium and not to the pubis. This process could not therefore be homologous with the "anterior process of the pubis" in Ornitischians. It seems, however, that the nature of the pectineal process is not the same in all birds. In the ostrich (*Struthio*) it is perfectly clear that the process belongs to the pubis, and BOAS claims that the same is the case in the other struthious birds. In carinate birds the authors generally consider that the pectineal process belongs to the ilium. In regard to the common fowl, however, the investigations of ALICE JOHNSON seem to afford good evidence that the *cartilaginous* pectineal process belongs to the pubis. Nevertheless the *osseous* process in the common fowl belongs to the ilium. I follow NAUCK (1938) when he says: "Was speciell die Verknöcherungsart des Proc. pectinealis bei Vögeln betrifft, sind aus ihr phylogenetische Schlüsse bezüglich seiner Herkunft unzulässig, nachdem . . . von BOAS (1930) die grosse Variabilität der Ossifikationsweise an grossem Material bewiesen wurde." When the ossification of the pelvis begins, the pelvis has already been cartilaginous for a comparatively very long time without any boundary limits between the three component parts. When the ossification centres of the three parts of the pelvis now meet, there is no security that they meet where there was in an early stage of development a boundary between the cartilaginous parts of the pelvis. It thus seems pretty certain, or at least not improbable, that the cartilaginous pectineal process primarily belongs to the pubis, whereas the osseous process of the ilium may secondarily replace the former. The former has a phylogenetical significance, the latter not. The homologies of the pelvic parts are set out in the following table.

Lizards	Saurischians	Ornithischians	Birds
lateral process of pubis	lateral process of pubis (*Diplodocus, Tyrannosaurus*)	prepubis (pubis: MARSH)	proc.pectinealis (prepubis: BOAS)
pubis	pubis	pubis (postpubis: MARSH, BOAS)	pubis (postpubis: BOAS)
ischium	ischium	ischium	ischium

From this table it seems to follow that the ornithischian pelvis and that of the birds are very similar, differing chiefly only in the size of the prepubis, which forms a very essential part of the pubis in the Ornithischians but is a for the most part rudimentary process in birds. As we cannot know anything about the ontogenetical development of the ornithischian pelvis, we must assume that it passes along the same lines as that of a primitive bird such as the ostrich. That is, at least, our only approach to an embryological explanation of the pelvis of the ostrich. In the preceding pages we have seen that the pelvis of the ostrich

can be explained as being derived from a pelvis like that of such a Saurischian as had a developed lateral process of the pubis, like many recent reptiles (lizards). It is from a reptile with such a pubis that the Ornithischians as well as birds must have sprung.

ABEL (1912) considered the pectineal process of birds to belong to the ilium. As consequently this process could not be homologous with the prepubis (pubis according to ABEL) of the Ornithischians, the latter was considered to correspond to the avian pubis. The postpubis of the Ornithischians thus had no homologon in birds. These conditions ABEL applied in the following way: "Dieser Vergleich ist vom ausserordentlichem Interesse. Er zeigt in klarer Weise, wie gleichsinnige Anpassungen bei verschiedenen Gruppen durch den Einfluss gleichartiger Lebensbedingungen — in diesem Falle die bipede Gangart — entstehen können, ohne dass homologe Elemente zum Aufbau verwendet werden. An der Stelle des Processus pectinealis der Vögel liegt bei den Dinosauriern das Pubis: und das Pubis der Vögel wird bei den Dinosauriern durch einen Fortsatz des Pubis, das Postpubis, vertreten. Das ist ein gutes Beispiel für *konvergente Anpassungen.*" As, however, the structures are homologous, there can be no question of a convergent development. Either the two pelves agree because of near relationship or because of parallel development. Later ABEL modified his conclusion, assuming that the agreement between the two pelves was due to parallel adaptation.

In spite of the very large measure of agreement between an avian pelvis and an ornithischian one, I must disregard this agreement as a token of a near relationship between the two groups, because many other important characters separate them distinctly, such as, for instance: the skull, the ascending process of the lower jaw, the teeth, the clavicle, the abdominal ribs, the ascending process of the tarsus, and many others. These differences are so considerable that we cannot imagine that the birds originated from the ornithischian stem. (Compare the discussion of these characters in HEILMANN 1926.) Thus there does not seem to be any other way of explaining the similarity of the ornithischian pelvis and that of the birds than to accept ABEL's later suggestion of a parallel development from a common primitive stem of Dinosaurs. This common stem had a saurischian pelvis with a well-developed external process of the pubis. In the line of the Ornithischians this process developed into a large prepubis simultaneously with the reversion of the pubis so as by degrees to lie parallel with the ischium. The other evolutional line, leading to the birds, either conserved the external process as a moderately developed primary (pubic) pectineal process or reduced it, substituting for it an osseous pectineal process of the ilium. The development of the avian pelvis may possibly, for all we know, have proceeded approximately along the lines indicated by the ontogenetical development of the pelvis of the ostrich, perhaps the most primitive of living birds.

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II. ON THE PHYLOGENY OF FEATHERS

1. SCALES, FEATHERS, HAIRS AND SPINES

Hairs and scales. The descriptions by WEBER (1927) and DE MEIJERE (1931) of the groups of hairs behind the scales in mammals made it very probable that the hairs are not entire scales but appendicular structures developing at or behind the posterior margins of the scales, in the very short posterior walls of the latter. Sometimes the hairs are also found to be situated on the dorsal surfaces of the scales, near their posterior margins (*Bradypus, Hystrix*). This disposition of the hairs in relation to the scales does not argue in favour of the hypothesis that the hairs are cylindrical scales sunk into the cutis. On the contrary, it speaks for the theory that they are parts of scales either as specializations of the rear margin of the scales or as sensory organs inherited from lower vertebrates (MAURER 1892, etc.); or such sensory organs as are found at the borders of the scales in certain reptiles (PREISS 1922). What interests us particularly in these two last-mentioned theories is that they both assume that the hairs are derivatives of scales and not the scales themselves. The position of the hairs in relation to the scales and their ontogenetical development are strong arguments for the non-identity between hairs and entire scales. The hairs may be organs on the posterior borders of the scales, but not modified scales.

Feathers and scales. The popular and generally accepted opinion seems to be that feathers are homologous to reptilian scales and have evolved from such scales. This development is most drastically expressed by HEILMANN (1926, p. 200): "By the friction of the air the outer edges of the scales become frayed, the frayings gradually changing into still longer horny processes, which in course of time become more and more feather-like until the perfect feather is produced. From wings, tail and flanks, the feathering spreads to the whole body." Similar views were held by PYCRAFT (1910). The scales covering the rear border of the future wing increased in length and "became also fimbriated and more and more efficient in the work of carrying the body through space." More elaborated is the theory of STEINER (1918), according to which the contour feathers are directly derived from scales, through the phylogenetical transformation of certain ridges on the scale to the different structures of the feather. However, he admits that the embryonic down does not correspond to the whole scale, but only to the posterior tip of it. "Tatsächlich hat auch SCHLEIDT nur bewiesen, dass die Embryonaldune nicht der ganzen Reptilschuppe homolog ist, sondern bloss einem Teile, und zwar stets der Spitze, etwas, das nach allem, was hier über die Natur der Neoptile gesagt wurde, als selbstverständlich erscheint. Um so eher ist die Annahme gerechtfertigt, dass die definitive Feder wirklich der

ganzen Schuppe homolog sei." (Op.cit., p. 467). This conclusion is logically not justifiable.

Already in 1895 DE MEIJERE had stated that there exist in birds groups of feathers situated behind scales like the groups of hairs in mammals. On the tarsus of *Strix flammea* scales of small (normal) size are present. Closely behind each of them is situated a group of three feathers. The mid-feather of these three is stronger than the others, just as is the mid-hair in a three-hair group in mammals. The groups of feathers on the tarsus of other birds such as pigeons and fowls are not so clearly comparable with the groups of hairs in mammals, as it seems probable that the tarsal scales in these birds are each composed of a number of primary scales.

In *Strix tengmalmi*, nestling, the tarsal scales are similar to those in *Strix flammea*, but only the mid-feather is present. This feather, however, was in many cases found *on* the posterior border of the scale. EWART (1921, p. 630) says: "As in feather-footed birds the feather papillae are from the first as distinct from the scale papillae as the hair rudiments are distinct from scales, there seems no escape from the conclusion that the foot feathers are not modified scales." On the other hand DAVIES (1889) came to a different conclusion. From the starting-point that the feathers are derived from scales, he thinks that all scales on the tarsus and the upper side of the toes were changed into small feathers, and that the scales now present on the tarsus and toes are secondary scales, which may have "in einigen Fällen als Verdickungen der Haut rund um die Ansatzstellen von Federn entstanden, in anderen wahrscheinlich als Verdickungen der Haut unabhängig von Federn." But this conclusion is only admissable if it is assumed that a feather represents an entire scale. Otherwise these big scales on the tarsus of birds are composed of primary scales and are secondary only in the sense that they are secondarily compound. In the opinion of STEINER just referred to, the neoptile is homologous only to the tip of the primary scale and the contour feather represents the rest of the scale. This opinion is in direct contradiction to the fact that the mesoptiles and teleoptiles all grow from the same feather follicle as the neoptile.

In an embryo of *Struthio*, more than half developed, there is at the dorsal border of the lateral apterium a zone with more or less delimited scales. On the posterior border of each of these scales a feather rudiment is developed. This rudiment is already sunk in a short feather-sac, the opening of which is located on the posterior tip of the scale and on its short posterior wall. If we compare it with less developed neoptile rudiments, before the formation of the feather sac (21-day embryo), it is obvious that this rudiment belongs to a scale territory of the skin which, however, in such young stages is not visibly delimited. This rudiment is the narrow cylindrical tip (filament) of the scale, which sinks into a feather sac. The pectoral callosity in the struthious birds is

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covered with scales, which are well developed, particularly in *Rhea*. At the margin of the scale-covered area the scales are combined with feathers, one at the top end of each scale.

The logical conclusion of these conditions must be that the contour feather cannot represent the whole reptilian scale, and that such theories as were mentioned above lack ontogenetical and morphological support. If scales did not develop into flying feathers, it is obvious that those Pro-avis reconstructions in which flapping with arms edged with long flat scales was the beginning of flight must be abandoned.

Feathers and hairs — homologous structures. The similar location of feathers and hairs at the posterior margins of the scales, when such are present, already affords strong evidence of the homology of feathers and hairs. This evidence becomes strengthened upon a comparison being made between the structure of the developing neoptile follicle and that of a normal hair. In both cases the structures issue from a root portion containing a pulp covered on the outside with formative cell-formations, from which the external parts of the hair cortex and marrow develop. The pulp is generally small and narrow in the hairs, very long and wide in the feather rudiment, where it extends from the base of the feather filament to its tip. In spines, which are modified hairs, the pulp is, however, very long. In the hair-follicle an inner root-sheath is present which dissolves when it has reached the level of the hair-sac glands. This root-sheath is composed of three distinct layers. In the feather follicle as well an inner root-sheath is apparently present, but it is closely attached to the cortex layers of the filament, approximately as is the case with the spine of the hedgehog. This agreement is found to be even closer if the radial arrangement of the cortex of the neoptile rudiment is compared with the radial arrangement of the spines of *Erinaceus*. In the coarse hairs of *Erinaceus* a radial structure of the cortex is diffusely discernible (DAVIES 1889, fig. 52).

There is, then, undoubtedly a certain measure of agreement between the hair and the hair follicle and the feather and the feather follicle. Nevertheless the earliest development of the two structures is so different that their homology might be doubted.

In birds the feather starts as a finger-like outgrowth of the epidermis at the posterior end of a scale territory or scale; in mammals the hair develops in a cylindrical ingrowth of the epidermis at a corresponding place. The result of the two methods of development is a close harmony of structure. In the case of the feather the formation of the feather rudiment comes first and is then followed by the formation of the feather sac. In the case of the hair the formation of the hair-sac starts the development, which ends with the formation of the hair proper. There is thus, in regard to the development of the filament, an acceleration of the formation of the feather proper and a retardation of the development of the feather follicle. Concerning the development of the hair

the chronology is reversed: retardation of the hair proper and acceleration of the development of the hair follicle.

Spines and feathers. DAVIES says (1889, p. 608): "Die auffallende Ähnlichkeit zwischen der Entwicklung des Stachels und der Feder und das ganz besonders mit Bezug auf die Bildung der Längsleisten auf den Wänden beider, veranlassten mich auf Professor Gegenbaur's Anregung hin, die Entwicklungsarten der beiden Gebilde einer eingehenden Vergleichung zu unterziehen." DE MEIJERE (1931) says: "Der vorübergehenden Ähnlichkeit der *Erinaceus*-Stacheln in ihrer Entwicklung mit einem Federkeim möchte ich keine grosse Bedeutung zuschreiben."

This agreement may, of course, be quite fortuitous, without any morphological or phylogenetical value, but it may also be of fundamental importance because the possibility is not excluded that hairs as well as feathers arose from spines located on the rear edge of reptilian scales. I should, however, pay but little attention to this agreement had not all spines that I had the opportunity of investigating shown more or less pronouncedly the same longitudinal ridges at the border between marrow and cortex. I have investigated these structures in *Echidna, Erinaceus, Ericulus,* and *Hystrix.*

The feather filaments of a bird's embryo are highly suggestive of spines. The ostrich embryo, which I saw hatching a few years ago in the Cape Province, looked soon after leaving the egg like a two-legged hedgehog, with all the filaments erect. A few minutes later all the filaments burst open and the spinous covering was transformed into a downy one.

Filament of Struthio. The filaments have been described so many times that it is not necessary to give a new description. We would only point out that the inside ridges of the cortex layer are basally divided longitudinally into two parallel bands ("ramogen pillars") by a lamella connecting with the cuticle of the filament. Apically this lamella separates from the cuticle. At its inner border pigment cells are accumulating. The lamella is a feather barb and the cell portions on each side of it are barbules. Outside the cuticle the filament is covered by the inner root-sheath. When the filament bursts the inner root-sheath and the cuticle are shed and the ridges become free, forming the barbs and barbules of the protoptile. The interior of the filament is occupied by the pulp, which extends to the tip of the filament. It contains blood vessels, of which superficial capillaries extend along each side of the ridges. At the basal end of the filament, the collar is the growth centre of the structure. From there new cortex material is added to the filament, and from there too the inner root-sheath is formed. It also includes an extreme basal portion (regeneration centre), from which the next feather arises.

Spine of Erinaceus. The spines of the hedgehog have been referred to many times as being similar to the filaments of birds. DAVIES (1889) made a thorough investigation of their development. The most characteristic feature of the spines

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in *Erinaceus* is the cortex ridges, which extend radially towards the centre of the spine. At the basis of the spine these ridges are broad and densely cellulated. Soon the ridges are divided into two, so that double the number arises (18—19). In each of these ridges there differentiates a horny lamella connected with the cuticle of the spine. Towards the tip of the spine the ridges gradually grow higher, and consequently the marrow of the spine becomes gradually narrower. The marrow contains blood vessels which send out branches in between the ridges.

Spine of Hystrix. The spines are much the same as in *Erinaceus,* though the ridges begin slightly more apically. In young spines the ridges are low, in older ones they grow higher and in the adult spines they almost meet in the centre of the spine. From the cortex layer a thin horny lamella enters each ridge.

Spine of Echidna. In *Echidna* the young spines consist of cuticle and cortical layer, the so-called layer of intermedial cells, and in the centre the pulp. The layer of "intermedia" cells is formed of parallel longitudinal ridges. These are heavily pigmented. The ridges extend from the base of the spine to near its tip. Apically they become more distinct, extending centrad. In the basal part of the spine the cortical layer is densely cellulated, while more apically the cells become scattered and horny.

Spine of Ericulus. The spines of *Ericulus* differ from those of the others as the longitudinal cortical ridges here are not very pronounced and are restricted to a short portion of the spine just apical to the root. The mesoderm papilla of the root has no connection with the marrow of the spine. It is separated from it by a section of the spine, where the cortex produces masses of cells, which form a broad septum in the spine. In this region of the spine indistinct ridges can be seen. The central cell mass of this region produces the peculiar alveolar marrow that fills up the central part of the spine. The cortex consists of three layers: cuticle, intermedial layer, and internal layer. The cuticle is a thin horny membrane with a few, tangential nuclei. The intermedial layer is the principal horny sheath of the spine. The horny substance forms longitudinal bands. The internal layer consists of a layer of variable thickness (thin in the apical part, thicker basally). It consists of circular horny fibres.

The presence of the radial structures in the spines may not perhaps have any very deep significance as they are the most natural structures in a cylindrical organ where strength in the material is desirable. Where, however, as in *Ericulus,* the question of strength is solved in another way, but where nevertheless a rudimentary ridge structure is present, we must assume that there are underlying reasons for the appearance of a ridge structure. This radial arrangement in the spines gains in interest when we consider that longitudinal ridges are also present in the hairs of *Erinaceus, Echidna,* and *Eremitalpa.* Perhaps the ancestors of mammals and birds were spiny creatures?

Change of hairs and feathers. When a hair has to be changed, the germ of

the new hair develops from the Malpighian layer at the bottom of the old hair follicle. The old hair at that time has already become free from the old follicle. A new hair bulb is formed and the new hair grows out in the old follicle, pushing the old hair before it (STEINLIN 1850, SCHWALBE 1893, MERKEL 1919). Some authors, however, maintain that the old papilla remains, revives and produces the new hair (KÖLLIKER 1850, SEGALL 1918).

When a feather is about to be changed, the germ of the new feather is likewise formed at the bottom of the feather follicle from the cells belonging to the stratum Malpighi of the old follicle. These cells are either merely a remnant of the old feather germ (BOAS 1931) or form a somewhat independent germ (STUDER 1878, KÜSTER 1905, BORNSTEIN 1911), which, however, originates from the old papilla. In the former case it can be said that the nestling down as well as the definitive feather develop from the same papilla, but it is obvious that the germ of the new feather must contain regenerative cells with a faculty not only of developing a new feather with a new growing centre but a new feather differing very much from the old one.

Even if it is difficult to demonstrate morphologically the presence of a new feather germ at the bottom of the neoptile, there must be such a new germ for the reason that the new feather is unlike the preceding one. This difference indicates either that the germ cells of the following kind of feather are physiologically radically changed or that they belong to morphologically different germs.

There is thus, fundamentally, but a slight difference between the changing of hairs in mammals and the changing of feathers in birds. In the birds the feather generations generally keep their continuity even after the new feather is fully developed. Owing to moulting, however, the continuity is broken.

Some research workers (JONES 1907, SCHAUB 1912) conclude from the mode of development of the different feathers from the same feather papilla that they belong to one and the same morphological unit. STEINER (1918) has contributed to this idea, referring to such often illustrated neoptiles as are the direct continuations of branches of mesoptiles or teleoptiles. He writes (p. 457): "Diese Kontinuität der Äste der definitiven Feder in jene der Erstlingsdune ist später vielfach beschrieben worden und wurde ebenso oft zur Darstellung gebracht. Dieser Zusammenhang ist jedoch nur verständlich, wenn wir annehmen, dass eine und dieselbe Federpapille in fortgesetztem Wachstum zuerst die Strahlen der Erstlingsdune und nachher jene der definitiven Feder bilde." The explanation of conditions such as those just mentioned is given by EWART (1921, p. 613): "In penguins the part of the epidermic tube which represents a calamus usually soon splits, with the result that the individual protoptile barbs are directly continuous with mesoptile barbs." We may similarly explain a case where a few of the mesoptile barbs split off from the "calamus" and carry

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small neoptile down whereas the remaining barbs are joined apically, forming the neoptile "calamus".

Consequently, apart from the uncertainty (probably the incorrectness) in itself of the assumption that one and the same feather rudiment forms the proto-, meso- and teleoptiles as a feather unit, the conclusion which STEINER has drawn from the fact that single mesoptile barbs may end in protoptile down is doubtful because this fact can be explained in quite a different way.

The following quotation from STEINER (p. 457) gives the basis for EWART's explanation: "KLEE, 1886, stellte fest dass die Spaltung der Papillenwände, welche die Strahlen bilden sollen, eine Unterbrechnung in der Gegend der Neoptilspule erfahrt, aber sofort weiter unterhalb dieser Gegend wieder einsetzt, um die Rami der definitiven Feder zu bilden." With this way of forming the "calamus" of the neoptile, a splitting process such as that described by EWART must always be a possibility.

A comparison between the change of hairs and feathers indicates that the two processes are fundamentally the same, in both cases consisting of a regeneration process. In the case of the hairs the process yields a new hair similar to the old one, in the case of the feathers the regeneration produces a new feather differing from the preceding one. Another point of difference is that the first feather hangs together with the following one, so that a chain of feathers arises. The comparison also tells us, however, that each type of feathers represents a separate generation of feathers, each of which has its phylogenetical value. STEINER says (p. 449): "Nach der ältesten Ansicht, welche Neoptil und Teleoptil als zwei getrennte Federgenerationen ansieht, und die speciell durch den Nachweis einer jedesmaligen Neubildung der Federpapille an Wahrscheinlichkeit gewinnt (BORNSTEIN), würde sich in der Tat die Deutung des Erstlingsgefieders als das primitive Federkleid des Vogelahnen ergeben." That is the standpoint I must take up on the question of the feather generations.

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2. FEATHERS ADAPTED FOR PROTECTION AGAINST COLD OR FOR FLIGHT?

Accepting the idea of the existance of different phylogenetically important generations of feathers in living birds, we come to the conclusion that the ancestors of birds, before they acquired the power of flight, were clad in a coat of down, which was developed for preserving a constant body temperature. This interpretation of the significance of the different feather generations does not satisfy STEINER (1918). As many authors did not find any fundamental difference between hair and feather rudiments "vielmehr nahelegten, dass beide als aus derselben genetischen Grundlage entstanden zu erachten sind, ist nicht einzusehen, warum aus demselben Organe bei gleichen Ursachen und Wirkungen nicht auch das Endresultat der Umformung dasselbe ist, d.h. warum im Dienste der gleichen Wärme enthaltenden Funktion, das eine Mal ein einfaches Haarge-

bilde, das andere Mal eine komplizierte Erstlingsdune enwickelt wurde." The fact that the hairs belong to mammals and the feathers to birds warrants the statement that the genetic basis for the development of hairs and that of feathers is not the same. It is, therefore, not in the least peculiar if the two animal groups have solved their problem of warmth in different ways but, nevertheless, use structures that are homologous. According to STEINER (p. 451), hair and feather cannot be parallel adaptations for the preservation of the body temperature: "Das Federkleid ist das eigentliche Flugorgan des Vogels. Der morphologische Bau der Vogelfeder ist derart an die Flugfunktion angepasst, dass es eigentlich selbstverständlich sein sollte, dass nur unter Berücksichtigung dieses Hauptmomentes alle ihre Eigentümlichkeiten erklärt werden können. Es frägt sich somit, ob nicht die Flugfunktion noch viel besser als die Wärmeschutzfunktion die Entstehung der Feder aus der Reptilschuppe zu erklären vermag, . . ."

The basis of such theories is, however, that the reptilian scale grows out caudad and is transformed into a contour feather. But such a theory cannot be maintained, because the contour feather arises at the bottom of the same feather sac as the down which precedes it, and this sac together with its feather rudiments represents the tip of a reptilian scale, not the entire scale. If the contour feather represented the scale proper, this would mean that the entire scale was buried in the bottom of the feather sac, and thus those real scales which have been described in birds would be secondary structures (DAVIES 1889, p. 607). Of course, this is the standpoint that must be taken up by those who see in the feathers of birds reptilian scales. However, the scales on the tarsi and feet of birds are pure reptilian scales and such scales are also described as existing in other parts of the body. Further, their earlier general presence on the body is clearly indicated by the quincunx orientation of the feathers in all bird embryos with a scale territory behind each feather rudiment. According to SCHLEIDT (1913) the nestling down does not represent the whole scale but only its tip. Accepting this view STEINER says: "Um so eher ist die Annahme gerechtfertigt, dass die definitive Feder wirklich der ganzen Schuppe homolog sei." But that does not agree at all with the embryological development of the definite feather.

Many workers have tried to explain in great detail how the reptilian scale was transformed into a contour feather. The more elaborate these explanations are, the more they are built up on a hypothetical basis. More hypotheses than facts are used as evidence for the explanations. For instance, the grounds for the hypothesis that the contour feathers are the primary feathers which have evolved direct out of reptilian scales are highly uncertain and probably incorrect.

Thus, the hypothesis that these "primary" feathers have developed from reptilian scales in order to serve the purpose of flight, must likewise be highly uncertain or incorrect.

If, however, we accept the theory that the down represents the primary

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feathers and the contour feathers were the last to be developed, we cannot accept the flight hypothesis, but must believe that the preservation of the body temperature was the primary function of the feathery clothing. The evidence upon which this last theory rests is chiefly the following:

(1) The down develops ontogenetically before the contour feathers.

(2) Scales connected with feathers are present on the feet, in the callosity area in Ratites, and on other parts of the body.

(3) The different kinds of feathers: proto-, meso- and teleoptiles (contour feathers) develop in a single feather follicle, probably from successively regenerating germs.

(4) The scale proper or a corresponding area of skin outside the feather follicle may be present simultaneously with the contour feather germ situated at the bottom of the follicle.

Besides the function of preserving the body temperature the feathers must have had another important function, viz. that of protecting the body from moisture. This function may have been correlated in some way with the development of the coupling together of, especially, the apical barbs to form a fairly dense covering, which could serve the two purposes, the hooked-together parts of the feathers, like roof-tiles, covering the downy part, thus forming an overcoat to give protection against cold and moisture. The primary function of the down was protection against cold, that of the contour feathers against cold and moisture. The last feather function to be developed was the function of flight, on the basis of the characters of the contour feathers.

This opinion regarding the development is quite contrary to that deduced by STEINER (1918). That author tried to show that all modifications of contour feathers and all warmth-preserving down were degenerated flying feathers. It seems, however, quite improbable that the very elaborately and ingeniously adapted pennae should have been the first step towards the contour feather and the downy clothing of birds. The reasons for this theory is, principally, that there are degenerate hooks present on the barbs in certain down feathers. As the hooks are most characteristic in the pennae their presence in down is said to be explainable only as a relic from the time when the downs were contour feathers. Now, this argument can be used equally well for the contrary theory, viz. that the contour feathers have developed from down and the pennae from contour feathers. STEINER has pointed out that the hook-apparatus of the quills is adapted for a pressure from below, whereas the contour feathers of the body are exposed to pressure from above. Nevertheless, the body-feathers are, fundamentally, built like the quills. To this STEINER (p. 460) remarks: "Hier muss aber ihre Funktion direkt als eine wiedersinnige genannt werden" and "Dieses Auftreten eines Merkmals in den Konturfedern des Rumpfes, welche bloss für die dem Fluge dienenden Schwungfedern begreiflich ist, kann nur durch die Annahme gedeutet werden, dass es sich um die persistierende Aus-

bildung eines Merkmals handelt, welches ursprünglich nur von den eigentlichen Flugfedern ausgebildet wurde." I think this interpretation is not quite correct, as the pressure on the contour feathers of the body is a double one, coming partly from the outside partly from the inside. The latter pressure is based upon the fact that the apical part of the contour feather rests upon an elastic down bed from which a counterpressure counteracts the pressure from the outside. The stronger this pressure is, the stronger grows the counterpressure and the stronger the coupling of the barbs with one another, in spite of the unsuitable arrangement of the barbules. As such elements as form the quill and other contour feathers are said to be already present in the protoptile, it seems impossible to imagine a gradual development from that stage to the contour feather and the quill. What the details of this development and its causes were it is impossible to tell. This evolution must, however, be easier to understand than the direct transformation of the reptilian scale into a quill.

In the struthious birds the feather clothing consists exclusively of down. No teleoptile generation is present. This is a highly significant statement, made by LOWE in his very important work "Studies and observations bearing on the Phylogeny of the Ostrich and its Allies" (1928). In this paper he says (p. 207): "There is not the slightest evidence of a teleoptylic phase of feathering comparable to that seen in the carinate flying bird," and continues that the assumption that the struthious down is, "derived from a proto-carinate volant feather, or in any way as a degenerate structure, seems to me completely unjustified by the evidence."

According to the theory that the struthious birds are birds that have degenerated from flying Carinates, the struthious down-feathers are degenerate carinate feathers. This view is supported (concerning the feathers) by the following observations of FÜRBRINGER (1888): In penguins, where the quills are reduced, the barbs are, apically, mainly devoid of barbules and but partly furnished with barbules with rudimentary fila and hooks. In Rhea not only have the barbs barbules, but the latter are provided with numerous though very small fila, some of which have knot-like ends approaching the hook-like fila in appearance. "Also bei *Spheniscus* kleine Flügelfedern mit spärlichen und reduzierten Wimpern und Häkchen, bei *Rhea* leidlich grosse Schwungfedern mit zahlreichen Wimpern und sehr vereinzelten und rudimentären oder abortiven Häkchen. Ich vermag keine wirkliche Grenze zwischen Beiden zu ziehen. Bei den Impennes dürfte die secundäre Rückbildung der Schwungfedern kaum zu bezweifeln sein; der Gedanke liegt sehr nahe, auch für *Rhea* (und die anderen Ratiten) einen ähnlichen Vorgang anzunehmen." STEINER (p. 454) confirms the observations of FÜRBRINGER concerning the feathers of *Rhea*: "An den tertiären Ästen, die bezeichwenderweise noch einen hochlamellenartigen Bau aufweisen, entspringen auf der unteren Leiste mehrere Häkchen, deren reduzierter Charakter allerdings stark zutage tritt, an deren Homologisierung mit den gleichen Gebil-

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den der Carinatenfeder nicht zu zweifeln ist." Concerning these conditions LOWE says (p. 205): "— — — in the barbule of the adult Rhea there is a considerable degree of specialisation leading to a condition of feather structure which neither approximates to down nor to the carinate barbule, but reflects in some degree certain details of the discontinuous barbule to be noted in the contour feathers of carinates in the juvenile phase. In the Rhea, therefore, we appear to have a condition of evolution of the feather more advanced than in all the other struthious forms. In the Ostrich, on the other hand, over-specialisation seems to have put the barbule definitely out of court from the point of view of any chance it may have had of developing into a volant barbule." CHANDLER'S (1916, p. 289) views are similar: "Like the Struthioniformes the Rheas show characters which might be construed as evidence of their being primitive in their flightless condition and of not being descendants of flying birds."

The arguments of FÜRBRINGER and STEINER are too weak and subjective to carry the weighty conclusions drawn from them. FÜRBRINGER was apparently uncertain how to interpret the rudimentary hooks, and STEINER says that the "reduced" character of the hooks is very conspicuous. (Why reduced? Why not progressively evolving?)

The evidence in favour of the original adaptation of the feathers for protection against cold or warmth and against moisture is, briefly, the following:

(1) The contour feathers are *not* homologous with the reptilian scales proper, in spite of the ingenious explanations offered of this homology.

(2) The proto-, meso-, teleo- and metaptiles all come from the same feather follicle.

(3) The sequence of these feathers is a kind of regeneration process comparable with the changing process of hairs.

(4) In struthious birds all feathers consist of down.

(5) In carinate birds down comprises the first developing feathers, which manifestly serve the purpose of regulating the temperature.

(6) When the contour feathers develop on the body, they serve the double purpose of temperature regulators and of protection against moisture.

(7) Finally the quills develop as an adaptation for flight. These points of evidence seem fully to justify the conclusion that down preceded quills in phylogeny and that the regulation of temperature was the primary function of the feather-clothing.

III. ON THE PHYLOGENY OF BIRDS

1. GENERAL SURVEY

In old days the birds were derived from Dinosaurs which were intermediate between crocodiles and birds (GEGENBAUR 1865, COPE 1867, HUXLEY 1868,

FÜRBRINGER 1888, and others). Others believed that Dinosaurs and birds had common ancestors (BROOM 1906, 1913) and that the points of agreement between these two groups were due to a similar adaptation without any close relationship. ABEL (1912) derived the birds from theropod Dinosaurs, in which the 4th and 5th fingers were more or less reduced. Among these Dinosaurs he also counted the Compsognathids. BOAS (1930) derived the Compsognathids and birds from Ornitischians.

Nowadays the birds are mostly derived directly from Pseudosuchians, such as Euparkeria, Ornitodesmus and others (HEILMANN 1926, STRESEMANN 1927—1934, PIVETEAU 1950), but not from the highly differentiated jurassic Coelurosaurs. VON HUENE's (1921 etc.) opinion was that the Pseudosuchians were the ancestors of the Coelurosaurs.

HEILMANN has summarized the chief points of agreement between the Saururae and the Coelurosaurs as well as between them and such Pseudosuchians as *Euparkeria* and *Ornithosuchus*.

As to *Archaeornis* and Coelurosaurs, agreement is recorded in the following parts:

(1) There are no essential differences in the bones of the skull, nor in the teeth.

(2) The vertebrae in general cannot be compared because those of *Archaeornis* are too badly preserved, but the neck vertebrae of *Hesperornis* agree essentially with those of *Struthiomimus*.

(3) In the sacral vertebrae there is a very close agreement.

(4) Ventral ribs are present in both groups.

(5) The sternum is not known in either group.

(6) The pelvis of the Saururae and Coelurosaurs are, according to HEILMANN, not essentially different, the pubis of bird embryos first extending downward, later on bending caudad.

(7) The agreement in the forelimbs is remarkable.

(8) The scapula and coracoid agree substantially.

(9) The femur has a 4th trochanter; tibia and fibula are exactly similar in the two groups. An ascending "intermedium" is present in both. The metatarsalia are in partial agreement.

Because of these points of agreement "it would seem a rather obvious conclusion that it is amongst the Coelurosaurs that we are to look for the bird-ancestor. And yet this would be too rash, for the very fact that the clavicles are wanting would in itself be sufficient to prove that these saurians could not possibly be the ancestors of birds." (HEILMANN).

The same author says that the points of agreement "denote less affinity to the Jurassic birds than an evolutionary tendency parallel to the development of some birds that have left off flying and in some respect are degenerate."

This remark obviously points to the struthious birds, which are generally

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considered to be carinate birds that have lost their power of flight and therefore became degenerate. As this theory is proving to be at any rate very doubtful (LOWE 1928), it cannot be used as evidence for another no less doubtful theory of a parallel development of Coelosaurs and birds, both of them descending from Pseudosuchians.

HEILMANN has summed up how a bird-ancestor should possess the following characteristics:

(1) The brain case must have been small.

(2) The skull and teeth should have the reptilian character of *Archeornis*, just as in Coelurosaurs.

(3) There should be a transverse bone present as well as

(4) a sclerotic ring.

(5) Amphicoelic vertebrae.

(6) Perhaps only two sacral vertebrae, as in bird embryos.

(7) A long tail.

(8) Ribs perhaps with uncinate processes. Ventral ribs.

(9) Clavicula and episternum present.

(10) Possibly a suprascapula present.

(11) Sternum present.

(12) Pubis still directed frontad, probably commencing to turn backwards. There is a "pubis- as well as an ischium-symphysis."

(13) Hand with 3—4, foot with 4—5 digits.

With the characteristics of the supposed bird-ancestor HEILMANN compares those of the Pseudosuchians, especially of *Euparkeria* and *Ornithosuchus*, and finds that they are very much the same. Let us make here a similar comparison, but include in it the Coelurosaurs as well.

(1) and (2) The cranial characters are, on the whole, the same in *Archaeornis*, Pseudosuchians and Coelurosaurs.

(3) The transversum is present in Pseudosuchians, absent in *Archaeornis* and Coelurosaurs. This difference HEILMANN explains in the following way: "The transverse bone became superfluous in birds; hence it has vanished in their case."

(4) In Pseudosuchians and birds sclerotic rings are present, in Coelurosaurs they do not seem to be known.

(5) Amphicoelic vertebrae are present in primitive birds and in the two reptilian groups.

(6) Perhaps there were only two sacral vertebrae in the Pseudosuchians, as in bird embryos, but *Archaeornis* probably had four. Perhaps but two sacral vertebrae were present in primitive Coelurosaurs as well, but in younger forms the number was greater.

(7) Tail long in all three groups.

(8) Ribs with processi uncinati present in Pseudosuchians and recent birds,

but not known in Saururae and Coelurosaurs. Ventral ribs present in all three groups.

(9) Clavicula and episternum present in Pseudosuchians. Clavicula present in Archaeornis and in modern birds. Episternum absent in birds, except in the embryonic ostrich. Clavicula and episternum not (preserved or) present in Coelurosaurs. HEILMANN points out the importance of the presence of clavicula and episternum in *Euparkeria* in the following way: "The shoulder-girdle of the Pseudosuchians consists of exactly the same elements as that of the birds and could easily change into same." The absence of the clavicula, episternum and sternum in Coelurosaurs is most probably only apparent. These parts were probably all present, but in such a form that they could not be preserved. They were either cartilaginous, membranous or merely present as rudiments in embryos. That they should have been absent altogether is highly improbable.

(10) Suprascapula present in bird embryos, now known in the two reptile groups.

(11) A sternum is unknown in the two reptile groups, well developed in birds, somewhat doubtful, however, in Saururae. However, it must have existed in them all.

(12) In the Pseudosuchians the pubis is still directed frontad, though commencing to bend backwards, and there is a pubis as well as an ischium symphysis present. I can scarcely imagine anything less birdlike than this pelvis. The ilium is very short and the pubis and ischium of each side are joined lengthwise from the tip of the pubis to the tip of the ischium with one or two foramina in the pubic part. It is true that the pubis of the bird embryo bends caudad, but, as we have seen in the preceding pages, the development of the pelvis of the ostrich passes a coelurosaurian state and not a pseudosuchian one. The pelvis of the Saururae can be derived from that of the Coelurosaurs but scarcely from a pseudosuchian one, as it retains the pubic symphysis, "while that of the ischia was lost." We must maintain that the pubis of the Saururae agrees very closely with that in, for instance, *Coelurus,* in which the lower parts of the pubes are joined, and with that of *Struthio* with a shorter part joining on to the pubes. Thus, the avian pelvis is more like that of the Coelurosaurs than that of the Pseudosuchians.

(13) The hand of the Pseudosuchians is not sufficiently known to be used for drawing a comparison with the birds. In *Euparkeria* parts of three fingers are found, which have been interpreted as the 1st—3rd according to the theropod pattern. They have been compared with the three fingers in the Saururae and birds, but as it has previously been shown that the fingers of the birds are the 2nd — 4th the comparison with the Pseudosuchians is very doubtful. In the primitive Coelurosaur *Procompsognathus* five fingers are present; the 1st and the 5th fingers are the weakest ones. In younger Coelurosaurs, the Compsognathids, there are three fingers and a rudiment of still one finger. As has been concluded

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previously, these fingers are the 2nd—4th and the rudiment of the 5th. Thus the hand of the Coelurosaurs is more birdlike than that of the Pseudosuchians. In the foot 5 digits are present; the 2nd—4th digits are stronger than the others.

The conclusion of this comparison is that the Coelurosaurs agree more closely with the Saururae than the Pseudosuchians do and that there is thus better evidence that the birds have a coelurosaurian than a pseudosuchian ancestry. As the Coelurosaurs are probably derived from Pseudosuchians, we arrive at the phylogenetic series Pseudosuchians — Coelurosaurs — birds. If we were to assume that the birds are direct descendants of Pseudosuchians, we should have to seek Jurassic connecting links between the Triassic Pseudosuchians and the Upper Jurassic Saururae. But no such links have hitherto been recorded.

2. "PRO-AVIS"

In the preceding pages evidence has been given for the old theory that hairs, mammalian spines and feathers are homologous structures and that they have a distinct relation to the hind margin of scales. In birds the feathers develop as structures at the tip of scales and are thus not transformed scales. Evidence has also been given for the anology between the regeneration of hairs and the formation of the different feather-generations. Finally, reasons have been advanced for the old theory that the feathers of the birds are in the same way as the hairs in mammals adaptations for preserving the body temperature.

All this evidence, together with the fact that the Saururae had feathers much as the carinate birds of to-day, seems to form a sound basis for the oft-stated conclusion that the ancestors of the birds were down-clad reptiles, belonging to a reptile stem issuing from Coelurosaurs more or less like Compsognathids (or to the Compsognathids proper).

According to these points of view *the Pro-avis was a down-clad reptile.*

As the Saururae and the Carinates are both characterized by having coverts as well as quills, it is very probable that the nearest common ancestors of these two classes of birds also had such feathers. In the struthious birds the feathers are all down-feathers. The coverts, which here are assumed to have preceded the quills, must thus have developed after the Ratites had separated from the common bird stem. The ancestors of the Ratites were probably down-clad. If, as is probable, they were primitive Compsognathids, these reptiles must also have had down-feathers (perhaps together with scales).

In the Saururae the 2nd, 3rd and 4th fingers are the functional fingers. Therefore the Pro-avis probably had these same fingers well developed but the 1st and 5th lacking or rudimentary. This was the case in the Compsognathids of the Upper Jurassic and the Cretaceous. Among the other Coelurosaurs the hand pattern is known to some extent only in the Upper Triassic *Procompsognathus.* In this genus the hand was, as far as we know, a normal 5-fingered hand. In

this hand the 1st metacarpale was much shorter than the others and the 5th a little shorter than the 4th, and much weaker. These metacarpalia were placed at a lower level than the others. The 3rd finger was the longest, though not much longer than the 4th. The number of the finger phalanges is not fully known, but the 1st finger probably has 2[1], the 2nd 2, the 3rd 3, the 4th 3 and the 5th 2 phalanges. From these statements regarding the hand of *Procompsognathus* we have fresh confirmation of the conclusion that the fingers of the Compsognathids are, as in the birds, the 2nd, 3rd and 4th, of which the 3rd is the longest one. The Phalange-formula of the hand of *Procompsognathus* according to the description given by VON HUENE (1921) probably was 2, 2, 3, 3, or 4, 2. The already somewhat rudimentary 1st finger of *Procompsognathus* only had to disappear and the 5th to become reduced to a metacarpale rudiment, and the essentials of the compsognathid hand pattern was brought into existence.

In the Upper Jurassic the procompsognathid-avian hand was developed. As the hand of *Coelophysis* (Upper Trias) already seems to have been a grasping hand, it would be very natural if the hand of later Coelurosaurs were a hand which could also be useful for grasping its prey. It could, however, also be useful for climbing, not necessarily in trees but at any rate on rocks.

The triassic Coelurosaurs such as *Procompsognathus*[2], and probably also *Hallopus* and *Saltopus*, were all bipedous animals, with a more or less reduced "hanging" hallux and reduced 5th toe. HEILMANN says: "For the foot of *Procompsognathus triassicus* proves beyond doubt that the hallux has been an oppositive hind-toe. Its ancestors must, therefore, have acquired it by climbing trees." The hallux of *Procompsognathus* was not oppositive but implanted rather high up on the inside of the tarsus, and its ancestors probably hand not "acquired it by climbing trees" but by running along the ground. The 5-fingered hand of *Procompsognathus* was certainly not specially adapted for climbing. There is no reason for believing any of the Triassic Coelurosaurs to have been arboreal. It would be more reasonable to consider them as terrestrial cursorial reptiles. In none of them were the metatarsalia fused as in birds.

The Coelurides of the Jurassic are very little known in regard to their feet. *Elaphrosaurus* had a somewhat birdlike but not a co-ossified metatarsus. In *Ornitholestes* we meet with a coelurid reptile with long free metatarsalia, a fairly well developed but not very strong hallux and a 3-fingered hand (fingers II, III IV) as in birds. The 3rd finger is much longer than the 2nd and 4th, and the 5th is rudimentary. The hand is very like that of the Saururae, with its abduced 2nd and very slender 4th finger. The mental picture I could gather from the literature on *Ornitholestes* is that of a bipedous cursorial reptile which used its long

[1] These numbers are quoted from the text in VON HUENE'S paper (1921) pp. 378—379. The text figure on p. 28 does not tally well with the text.

[2] *Ammosaurus* had not prolonged metatarsal bones and was probably not bipedous. Its hand was differentiated like that of a Pseudosuchian or Theropod.

anterior limbs and strong claws for catching and holding its prey rather than for climbing in trees. Nor do the posterior limbs seem to be adapted for climbing: the long legs and the backward or medial direction of the short hallux, which was raised above the ground, were not well suited for that purpose. The limbs of *Compsognathus* were much the same, though the 1st toe was more rudimentary, situated above the level of the other toes, and the fingers shorter. The comparatively short fingers of this carnivorous Coelurosaur were provided with strong claws, with which the animal could catch and hold its prey. The Cretaceous *Struthiomimus* was a running, long-legged bipedous animal with long, fairly strong fingers, which must have had a grasping function. As the animal had no teeth, it was not a beast of prey but probably a vegetarian like the ostrich, and its comparatively long arms could then be used for pulling down branches for browsing upon leaves or fruits.

These comparisons, incomplete though they are, seem to indicate that the Coelurosaurs were chiefly cursorial reptiles, the younger of which had an anterior limb skeleton fundamentally agreeing with that of the birds. This fact may perhaps indicate that the perculiar character of the hand had something to do with the development of feathers and the acquisition of the power of flight. In the preceding pages we have concluded that those Coelurosaurs from which the birds derived were down-clad forms. When comparing the hand of *Ornitholestes* with that of *Archaeornis* it is difficult to avoid the thought that it was the developing wing feathers that caused the lengthening of the fingers in *Ornitholestes* and that in the Coelurosaurs there were the makings of an avian wing. These wings may have been developed more or less as those of the ostriches with large-sized down instead of quills, and could have served as a flapping apparatus or as aircraft wings, easing the body for swift running. The pneumaticity of the bones of the Coelurosaurs also helped to ease the body. From such Coelurosaurs the Ratites could easily have been derived. From cursorial Coelurosaurs arboreal types could arise through the development of an oppositive hallux. ABEL presumes that such a hallux "auf ein arboricoles Leben der Vorfahren zurückzuführen ist" and thinks that it would be to violate the law of DOLLO to presume the possibility of a small hallux developing into a larger one. As long as the hallux was small, the ancestors of the birds were not well adapted for arboreal life. They could, of course, jump up and settle down upon big tree trunks but not on weaker branches, for which the oppositive hallux would be necessary. For climbing big tree stems the hind limbs of the Coelurosaurs must have been very poorly adapted with the small 1st toe raised above the level of the other toes, as in the Limicolae of to-day. As there is no Coelurosaur with a real oppositive hallux known to us at present, we must assume that this sort of a hallux arose during phylogeny, perhaps somewhere between the point of issue of the Ratites and the common ancestor of the Saururae and Carinates, when the teleoptile (covert) feathers replaced the metaptile down. The develop-

ment of the teleoptile coat was the foundation of the birds' ability to fly. From such feathers the quills arose and thus the evolution leading to flying birds could begin. But before that there was a long period of preparation for the development of wings usable for flight. In that period the highest power of flight was confined to facilitating the bird's running by flapping the down-clad anterior limbs, on which ultimately certain large-scale down located as remiges (quills) developed, as previously explained. FÜRBRINGER, who, however, interpreted the struthious birds as being degenerate Carinates, says (1888, p. 1505): "Dadurch würden die *bekannten Ratiten* also als sekundäre, von *primitiven Carinaten ableitende Typen* aufzufassen sein und es muss sehr wohl zwischen *ihnen* (Deuter-Aptenornithes) und jenen noch gänzlich unbekannten *primitiven Ur-Ratiten* (Prot-Aptenornithes) unterschieden werden, welche nothwendig die Vorfaren aller Vögel gewesen sein müssen und eine geräume Zeit hindurch, bevor das Flugvermögen zur Ausbildung kam, am Ende der palaeozoischen und am Angange der mezozoischen Aera als die einzigen Vertreter der Vogelklasse lebten."

The previously drawn comparison between the Compsognathids and *Archaeornis* has given as a possible result that if the Saururae had not been found with feathers, they would probably have had to be ranged among the Compsognathids or the latter among the birds. As already explained, the Coelurosaurs were probably down-clad. Why not, then, *interpret the Coelurosaurs as representing* FÜRBRINGER'S *"Ur-Ratiten," from which the Ratites and the flying birds developed?*

If this theory proves acceptable, the question of the Pro-avis breaks up into two parts, viz. into the question of a pro-ratite and a pro-carinate bird. The former had but to continue the life of its ancestors, using their legs and the flapping wings as propulsive organs. The latter had to acquire covert feathers and grow some of them into quills. This Pro-avis in the restricted sense of the word (Pro-carinate) would be like the traditional Pro-avis, with this difference, however, that instead of scales the bird had coverts and thus used its covert-feathered wings instead of scaled wings as a parachute during the first attempts at real flight.

The assumption of a pre-struthious ancestor of the struthious birds does not accord with the prevailing acceptance of FÜRBRINGER'S interpretation of the Ratites as degenerate Carinates. The critical remarks of LOWE, however, seem to have shaken the foundations of (OWEN-)FÜRBRINGER'S theory. Later I propose to consider this question at some length.

One difficulty for the preceding theory of the phylogeny of birds is the following. The fingers are but little reduced in the Saururae but strongly reduced in the Ratites and Carinates. The Saururae and the Carinates have teleoptile remiges, but the remiges of the Ratites consist of metaptile down. This difficulty cannot be surmounted in any other way than by assuming that the reduction of the fingers began independently in Carinates and Ratites or that

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the Carinates and Saururae acquired their coverts and quills independantly. The latter possibility seems to be less acceptable than the former. This difficulty would easily be overcome if we were prepared to accept FÜRBRINGER's theory of the carinate origin of the Ratites, but that we are not prepared to do.

3. THE TIME FACTOR

One objection against the phylogenetical development of the birds we have just sketched is based upon the fact that the first bird and the most birdlike of the Coelurosaurs were more or less contemporary, both belonging to the Upper Jurassic. If that is true it is impossible that the birds could have descended from them. Their ancestors must have been considerably older, how much older we cannot say. Assuming, however, that the Coelurosaurs of the Jurassic were already covered with down, the time elapsing from that period to the appearance of the ancestors of the flying birds can be estimated to have been much shorter than if the birds were to be derived from scaly reptiles, as most students of birds assume. As birdlike Coelurosaurs were probably present from the Upper Trias *(Podokesaurus)* and throughout the whole of the Jurassic, there must have been plenty of time to replace down with contour feathers and transform certain contour feathers into teleoptile remiges.

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4. "PRO-CARINATAE"

Our interpretation of the phylogeny of the birds does not agree with the generally accepted theory of the descent of the Ratites from flying birds of the carinate stem, which had lost their power of flight and therefore became in many respects degenerate. The idea of the struthious birds as degenerate Carinates, started by OWEN (1886), was worked out in detail by FÜRBRINGER (1888), T. J. PARKER (1892), GADOW (1891—93), BEDDARD (1898), and others. But there are a number of authors who have arrived at the conclusion that the Ratites have not developed from flying birds but from a stem of primitive birds from which the Jurassic birds and the Carinates also originated. Such authors are LUCAS (1916), CHANDLER (1916), HEILMANN (1926), LOWE (1928), FRIANT (1945—46).

If we are looking for the characters of these Pro-carinates, we can only ascertain that they were more or less well developed flying birds. From such birds the Ratites should have arisen, through secondary degeneration of their power of flight. "Dabei gehen aber gewisse *primitive* Züge in den verschiedenen Organsystemen, welche sie mehr noch als die lebenden Carinaten gewährt haben, zum Teil auch die beträchtliche Körpergrösse, welche einen längeren Entwicklungsgang als bei den kleinen Vögeln voraussetzt, zugleich an die Hand, den Beginn dieser reductiven Vorgänge in eine *sehr frühe Zeit*, vielleicht in den

Anfang der mesozoischen Periode zurückzuverlegen, wo alle Vögel sich noch auf einer relativ primitiven Entwicklungsstufe befanden und wo die junge Flugfähigkeit der Ur-Carinaten mit ihren noch wenig fixierten Skelet- und Muskel-Configurationen eine gründlichere Rückbildung ermöglichte, als z.B. bei jenen später fluglos gewordenen Vögeln."

"Dadurch würden die *bekannten* Ratiten also als sekundäre, von *primitiven Carinaten abzuleitende Typen* aufzufassen sein und es muss sehr wohl zwischen *ihnen* und jenen noch gänzlich unbekannten *primitiven Ur-Ratiten unterschieden* werden, welche notwendig die Vorfaren aller Vögel gewesen sein müssen und eine geräume Zeit hindurch bevor das Flugvermögen zur Ausbildung kam, am Ende der palaeozoischen und am Anfange der mesozoischen Aera als die einzigen Vertreter der Vogelklasse lebten."

Thus, the power of flight of the pro-carinate birds was already established very early, perhaps at the beginning of the mesozoic aera. If we compare with this idea the fact that *the birds of the late Jurassic* were probably poor flyers, then some doubt about the early development of flight in the pro-carinate birds must be justified.

Still more must we question the idea that these pro-carinate flyers were already differentiated into long-winged sailing birds and short-winged flying birds. If there is already any doubt about the power of flight of the pro-carinate birds, then our doubts about the development of long-winged struthious birds from long-winged pro-carinates and short-winged from short-winged ones must be all the stronger. If the Pro-carinates had already evolved in the Triassic era, they were certainly not flying birds at all. In such circumstances any discussion about the flying ancestors of the struthious birds would be meaningless. Instead the conclusion would be that the struthious birds branched off from the carinate stem before they had acquired the power of flight, a conclusion that agrees with those arrived at in the preceding chapters. The only way in which we can obtain some information about these flightless birds (Pro-aves) is by making a comparison between *Archaeopteryx*, the struthious birds, the Carinates and the Coelurosaurs. Such comparisons have already been sufficiently made above. It only remains to point out here that the struthious birds are primitive in a great many characters. As the question of the primitivity of the struthious birds is one of firstclass importance, we shall consider it at some length. GADOW (1891—1893) has made, after FÜRBRINGER and W. K. PARKER, an enumeration of the chief characters of the Ratites, and we will follow his list.

5. PRIMITIVE AND PSEUDO-PRIMITIVE CHARACTERS

GADOW divides the characters of the Ratites into 3 groups: (1) Primitive characters present in the Ratites only. (2) Primitive characters also present in the Carinates. (3) Pseudo-primitive characters.

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(A) *Primitive characters present in Ratites only.*

(1) The well-developed copulatory organ, which is connected by muscles with the skeleton.

(2) The complete procoracoid in *Struthio*. This character is graded, as it is more or less rudimentary in other struthious birds.

(3) The lack of down is said to be primary in the Ratites. The fact is, however, that all the feathers of the Ratites consist of down. After having carefully considered the question of the feathers in the Ratites, LOWE (1928, p. 207) says: "Strange, therefore, as it may appear, all the feathers borne by the adult Ostrich or by any other struthious form, whether they are situated on the wing, tail or body generally, are nothing more than down, or modified down. There is not the slightest evidence of a teleoptylic phase of feathering comparable to that seen in the carinate flying bird, so that to regard the struthious wing-feathers as secondarily derived from a proto-carinate volant feather or in any way as a degenerate structure, seems to me completely unjustified by the evidence." (Cf. the special chapter above concerning the feathers in birds).

(4) The presence of only one series of lower tectrices in *Struthio* to be a primary condition. WRAY (1887), has, however, stated that this series originally belonged to the upper side of the wing and has moved over to the lower side. The primitivity of this condition thus seems to be doubtful. It seems more probable that a naked lower side of the wing is more primitive.

(5) Traces of a claw on the ulnar finger in *Struthio* and *Rhea* undoubtedly represent a primitive condition.

(6) The undoubtedly primitive conditions of the intestinal tract.

(B) *Primitive characters of Ratites also occuring in Carinates.*

(1) Typical "Nestflüchter".

(2) Neoptiles in *Struthio*. The neoptiles are fundamentally the same in all birds.

(3) Quintocubitalism (or eutaxy) is generally said to be primary in birds. According to STEINER (1918) the aquintocubitalism or diastataxy is primary.

(4) Compound rhamphotheca, like that in Crypturi, Steganopodes and Tubinares.

(5) "Holorhinae nares imperviae", that is, nares with a complete septum.

(6) The conditions of the basipterygoid-processes, also in Crypturi.

(7) One single proximal quadratum-articulation, also in Crypturi and *Ichthyornis*.

(8) Vomer large, extending far caudad. Not in *Struthio*.

(9) Structure of palatine bones, also in Crypturi.

(10) Coracoids separate from one another.

(11) Incisura ischiadica. Also in *Apatornis,* Crypturi, and *Hesperornis.* Foramen ischiadicum in *Rhea* and *Dromaeus.*

(12) Hypotarsus simple, primitive.

(13) Femoral muscles primitive.

(14, 15) Toe-flexors in the 4-toed forms and number of toes in *Apteryx* and *Dinornis* primitive.

(16) Coeca primitive.

All these characters, which the Ratites have in common with at least some carinate birds, must be regarded as being more or less primitive. The third group of characters, the "pseudo-primitive" ones, includes the characters most important for judging the nature of the Ratites as compared with the Carinates.

(C) *The pseudo-primitive characters.*

(1) The absence of apteria is said to be a pseudo-primitive character. Now, the embryos of all struthious birds generally have three apteria: a spinal, a mesogastric and a lateral trunk apterium. LOWE (1928), who made this statement, gave good reasons for considering that state of things to be primary and that the "pterylar coat of the existing Struthiones represents a primitive covering, which existed in birds in general before the acquisition of flight."

(2) Absence or enormous development of "aftershaft." LOWE's, and especially EWART's (1921), extremely interesting investigations into the feathers of birds make it very certain that the aftershaft is a primitive structure of the bird's feathers.

(3) Absence of oil-glands. GADOW (1891—1893) considers this to be a character that indicates degeneration, probably in correlation with the structure of the tail-feathers and of the pygostyle. It is true that *Struthio, Rhea, Dromaeus,* and *Casuarius* have no oil-gland, but *Apteryx* has. This latter fact seems to indicate that the gland is a primitive organ in birds, already present in primitive struthious birds. In recent struthious birds its absence may be due to a very early reduction, as it is not present, for instance, in embryos of *Struthio.* It is, however, not possible to decide with any certainty as to the primitivity or non-primitivity of the oil-gland. Those authors who derive the Ratites from the Carinates say that, when the carinate feather clothing became degenerate in the Ratites, the oil gland disappeared simultaneously, except in *Apteryx.* I cannot express any opinion about this assumed fate of the oil gland, but can only suggest that the very different mode of life of the large species on the one hand, and the small *Apteryx* on the other, may sufficiently explain the loss of the gland in most Ratites and its preservation in *Apteryx.* Thus I cannot offer any strong evidence for my personal opinion that the ancestors of the birds had an oil gland, which disappeared in the Ratites except in the *Apteryx* line of their development.

(4) The absence of the pygostyle has been said to be pseudo-primative because

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a pygostyle-like formation had been found in a specimen of *Struthio* as well as in *Apteryx*. LOWE considers the general absence of the pygostyle in the Ratites to be a secondary character. I think that the fusion of the "lost" tail-vertebrae may be correlated with the shortening of the tail, which took place perhaps at an early point of time independently in Ratites and Carinates. The question of the pygostyle will be treated separately below.

(5) The absence of a sternal keel is said to be pseudo-primitive. As this question is of the greatest importance for the phylogeny of the birds, it will be treated separately.

(6) The question of the scapula and coracoid, which is also one of great importance, will also receive separate treatment.

This enumeration of the characters of the struthios birds undoubtedly testifies to the very primitive nature of these birds. The fact that many of the Carinates have primitive characters like those in the Ratites is quite natural, as both groups have undoubtedly developed as branches from the same stem.

6. ARE RATITAE DESCENDANTS OF PRO-CARINATAE OR NOT?

FÜRBRINGER (1888) and T. J. PARKER (1892) have summarized the grounds on which they base their interpretation of the struthious birds as being birds which have, at an early stage of their development, been flying birds. "Dies wird bewiesen vor allem durch das Skelett der vorderen Extremitäten, das mit seiner Reduktion der Handwurzelknochen und der Synostosierung der Metacarpalia ein typisches Flügelskelett ist, durch die Anwesenheit der Schwungfedern und grossen oberen Deckfedern und anderes. Der Verlust des Flugvermögens hat sich bei den „*Ratiten*" offenbar zwangläufig aus der bedeutenden Steigerung der Körpergrösse ergeben. Er zog nach sich: Verkrümmerung der Brustmuskulatur, Zurückbildung der Crista sterni, die dieser zum Ansatz dient; synostotische Verbindung von Scapula und Korakoid zur „Korakoscapula", starke Vergrösserung des Korako-Scapular-Winkels, Schwund des Akrokorakoids der Scapula; Zurückbildung der Furcula biss zum völligen Schwund; Verringerung der Grössenunterschiede zwischen Radius und Ulna; Vereinpackung des Handskeletts und der Flügelmuskulatur; Umbildung der Schwung- und Steuerfedern zu Schmuckfedern oder völlige Rückbildung dieser Federn; fortschreitende Auflösung des Pygostyls in seine Wirbelelemente; kräftige Entwicklung der Beine; Schwund der Pneumatisation der Röhrenknochen allerfalls mit Ausnahme des Femur; Schwund der ursprünglichen Differentierung der Pterylose in Federfluren und Federraine; Schwund des bei den Flugvögeln durch Zellfortsätze bewirkten Zusammenhalts der Federfahne; Herabsinken des Federbaues auf den Typus der „Halbdune", woraus sich als weitere Folge ergab: keine Differentierung des Federkleides in Konturfedern, Pelzdunen, Fadenfedern und keine Strukturdifferenzen zwischen Basalteil der einzelnen Federn.

Das zerschlüssene Gefieder machte ein Einfetten entbehrlich: daher Schwund der Bürzeldrüse (ausser in Apteryx)." (STRESEMANN in KÜKENTHAL's Handbuch).

We shall now consider some of these arguments for the secondary loss of the power of flight in the Ratites.

(1) *The reduction of the wrist bones and the synostoses of the metacarpalia* are said to afford strong evidence to justify the theory that the Ratites were once flying birds. How can that be? *Archaeopteryx* and *Archaeornis* were undoubtedly flying birds even if their power of flight was not perfect. But their wrist was not much more reduced than that of the ostrich, and the metacarpalia were not fused with one another. In addition, the number of phalanges was less reduced in the Saururae than in the ostrich, the phalangeal formula of which, however, comes near to that of the Saururae. If the theory of WRAY (1887) about the 4th finger in *Struthio* is correct, this finger has two additional phalange rudiments on the tip of its second phalange. If we count these rudiments as well, the phalangeal formula of the ostrich is the same as that of the Saururae. In the other Struthiones a retrogression of the hand is more obvious, whereas the retrogression of the hand of the Ratites in general was not directed so that the hand ultimately came to be adapted for flight. The conditions in the Saururae show that retrogression of the hand was not a condition for acquiring volant power and that the development of volant feathers thus probably preceded the retrogression of the hand skeleton. If this conclusion is correct and the volant feathers have evolved from the same source in all birds, the retrogression of the hand must have been based upon other factors than the appearance of volant feathers. After the latter's appearance the retrogressive hand was adapted to provide strong support for these feathers. The phalangeal formula of the hand in *Opisthocomus* is (—, 2, 3, 2,—), and the same formula occurs in *Struthio*, if we disregard WRAY's theory. It is, then, obvious that the hand of the Carinates as well as that of *Struthio* can have arisen from a hand like that of the Saururae. In the Carinates the somewhat retrograde hand was adapted for an important use, in the Struthiones the retrogression, beginning with rather primitive conditions as in *Struthio*, continued with a degeneration of a type that could not have followed upon a flying status. In the Saururae and Carinates the flying feathers developed, but in the Ratites the feathers reached their highest stage of development only as prepennal down instead of the pennae of the Carinates.

The first Carinates to develope were probably as bad flyers as *Opisthocomus* (and the Saururae), and the Pro-carinates (if they ever existed) were not flyers at all but exclusively cursorial or climbing birds.

(2) *Degeneration of (pectoral) muscles.*

BEDDARD (1898) says that there is but little, if any, evidence of degeneration of the musculature of the hand in Struthio, where the musculature is less degenerate than in the Carinates. "It seems to be conceivable that the ostrich branched

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off from the avian stem before the power of flight was perfectly established" (p. 501).

LOWE (1928) laid stress upon the fact that there is no patagium developed either in the embryo or in the adult of *Struthio, Rhea, Casuarius,* and *Dromaeus.* In *Apteryx oweni* he did not find any patagium, but in *Apteryx bulleri (australis mantelli?)* T. J. PARKER (1892) described and illustrated patagia. It must, however, be remarked that, according to LOWE, with a maximum flexion of about 45° of the antebrachium against the humerus the looseness of the skin of the wing must produce a patagium-like fold. The question of the presence or absence of a patagium is of importance for judging the question of the degeneration of a patagial musculature that may perhaps have been present originally. FÜRBRINGER (1888), T. J. PARKER (1892), BEDDARD (1898) and many other writers have named a number of patagial muscles which are said to have disappeared concurrently with the disappearance of the patagium when the struthious birds lost their power of flight. It is curious — if that is true — that those struthious birds in which the forelimb is most degenerate should have preserved the patagium, whereas those in which the wings are less degenerate should have lost it, and with it also its muscles. The evidence indicates that the Ratites never had any patagium, nor any purely patagial muscles.

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LOWE's investigation into the pectoral musculature is most interesting and affords good evidence against the theory that the pectoral musculature of the Ratites is of a degenerate carinate type. On the contrary, all the features of this musculature speak for its being primitive. I cannot go into details, but have to refer the reader to LOWE's paper (1928).

(3) *Crista sterni.*

The generally accepted opinion is that the sternum of the Ratites is a carinate sternum, the crista of which has disappeared as a consequence of the loss of volant wings and the reduction of the pectoral wing musculature. We shall now consider the grounds for that theory.

FÜRBRINGER says (1888, p. 1499): "Die Crista sterni wird neidriger und niedriger bei den grösseren Formen der Tubinares, Steganopodes und Accipitres, bei zahlreichen Rallidae, Mesites, Menura, Atrichia, Dididae, Cnemiornis, Stringops, Apatornis, um schliesslich bei den letzterwähnten Gattungen in partiellen Schwund zu treten oder ohne Grenzen in die Sternalfläche sich abzuflachen; dass damit nicht selten eine beträchtliche transversale Wölbung des Brustbeines coincidiert, die cristale Rückbildung einigermassen compensierend, wurde bereits erwähnt."

And FÜRBRINGER continues: "Denkt man sich diesen Reductionsprocess weiter fortgesetzt, so entsteht das ratite Sternum, sei es hochgewölbter (Casuarius, Struthio und namentlich Rhea), sei es in mehr flacher Form (Hesperornis, Apteryx, Dinornitidae)." If we follow FÜRBRINGER's suggestion we would

certainly arrive at a sternum without a crista, but that would be a sternum with only superficial likeness to a struthious sternum. But FÜRBRINGER has laid stress upon the so called protuberantia present in *Struthio* and especially in *Rhea*, which suggests a broadened and flattened out crista: "Und wer das Sternum von Rhea mit seiner transversalen Wölbung, seiner Protuberantia und seinen seitlichen Eindrücken zu beiden Seiten der Protuberantia genauer vergleicht und betrachtet, der kann sich kaum der Auffassung entschlagen, dass hier ein aus einem carinaten Brustbein hervorgegangenes Gebilde vorliegt, wird aber schwerlich die Meinung begründen können, dass es sich um eine phylogenetische Jugendform handele, welche im aufsteigenden Entwicklungsgange zur Cristabildung sich befindet."

The "protuberantia sterni" are thus considered to represent probably the crista of the Carinates. The reasons for that interpretation seem to be rather weak, and GADOW says (1891, p. 955), "Ob diese von FÜRBRINGER bei mehreren noch jungen Exemplaren von *Struthio* und *Rhea* abgebildete und Protuberantia sterni genannte Leiste wirklich als Rest der bei den Ratiten sonst allgemein unterdrückten Crista sterni aufzufassen ist, bleibt unentschieden." In spite of this expressed doubt about the protuberantia as representing the keel of the sternum, GADOW says (1893, p. 94): "Ich kann das Fehlen des Kieles nur als ein durchaus sekundär erworbenes, also pseudo-primitives Merkmal auffassen. Ebenso urteilen FÜRBRINGER und T. J. PARKER."

The protuberances, when present, lie in *Struthio* at the hind edge of the sternum as two swellings, one on each side of a rather deep pit. In *Rhea* they are found a little more in front of the posterior edge of the sternum. In two skeletons of *Rhea*, one young and another quite old, and in one of an old *Struthio* the present writer found no trace of any protuberances.

There are thus already contradictions in regard to the occurrence of these protuberances; sometimes they occur (in young specimens), sometimes not. It seems necessary, therefore, to resort to embryology as the only way by which we can hope to arrive at an acceptable result.

In a carinate bird (fowl) the development of the sternum is described in LILLIE & HAMILTON's "Development of the chick" (1952) as follows. "The sternum arises from two bilateral mesodermal plates, which lie at first in the dorso-lateral wall of the thorax at the posterior end of the pericardial cavity. The two sternal rudiments lie immediately below and behind the mesoderm which forms the coracoid and are continuous with it; they are however, entirely distinct from the sternal division of the ribs. The sternal plates move from their dorso-lateral position in the ventral wall of the thorax. This movement is part of a general expansion and growth downward of the tissues of the upper part of the thoracic wall, accompanied by degeneration of the original mesenchyme of the ventral body wall. As their ventral edges approach in the midline, each sternal plate bends sharply to form an upper horizontal portion (the

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sternum proper) and a lower vertical part. These latter unite, beginning at the anterior end, to form the dorsal part of the keel. . . .". "The ventral extension of the keel is formed by the migration of chondrogenic cells from the sides of the opposed sternal plates downward into the degenerate mesenchyme of the mid-ventral line."

Miss LINDSAY'S (1885) investigation of the development of the sternum of *Struthio* does not provide any evidence for the theory of an earlier existing sternal keel. She does not mention any downward-bending of the borders of the sternal plates such as might foreshadow a developing or degenerating keel. Nor has she seen any protuberances in the young developmental stages.

In a 2.5 cm. long sternum of *Struthio,* which had been studied by LINDSAY, FÜRBRINGER found that the cartilaginous sternal plates had along their medial borders a somewhat ventrad protruding longitudinal elevation (list), corresponding to a longitudinal groove on the inside (dorsal side) of the sternum. GADOW says about this elevation: "Es ist somit eine Bildung, welche einer sich eben entwickelnden Crista sterni wenigstens analog erscheint." Comparing this with the description of the development of the carinate crista as quoted above, we cannot find any agreement at all. As, however, I have in my collection an ostrich embryo with a sternum about 2.5 cm. long I have closely studied it. A description of this sternum follows below, it being located in its place in the series of sterna now to be considered.

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In 9—13 cm. stages of Struthio australis the sternal plates are triangular. They are more or less separated, but their anterior ends successively approach one another. The posterior ends also are growing towards the mid-line. Between the plates the linea alba runs frontad to reach the anterior end of the sternum. The anterolateral and the posterolateral corners are well developed. There is still no xiphisternum present.

In the 15-day stage the sternal plates have met frontally but still diverge caudad. In the band of tissues between the two plates the linea alba runs frontad. This band of tissues broadens caudad and ends in a short, quite blastematic xiphisternum. The posterolateral processes have grown out, so that their ends lie on a level with the caudal end of the xiphisternum. The length of the sternum is 0.8 cm., its breadth 0.6 cm.

In the 20-day stage the length of the sternum is 1.2 cm., its breadth 1 cm. The sternal plates have met each other in the mid-line approximately within the frontal half of the sternum. There is, however, a very conspicuous suture between them. In the caudal half the medial borders of the plates diverge considerably and run out into the posterolateral processes. In the triangular space between the posterior ends of the sternal plates the metasternal part of the sternum sends in a fairly well-defined triangular, somewhat elevated process. The xiphisternum proper is somewhat attenuated caudally.

In an older embryo, the sternum of which measures 2.5 cm. in length and 1.9

cm. in breadth, the somewhat elevated anterior part of the metasternum has become enclosed between the two sternal plates, of which the posterior parts have become somewhat swollen and widened mediad, so that the anterior part of the metasternum is almost entirely surrounded by the sternal plates. The anterior part of the metasternum, however, continues caudad as a bandlike portion, which seems to have been pressed down below the level of the plates[1]. This part passes into the xiphisternum, which ends in a small cartilaginous nodule. The longitudinal lists at the medial lines of the sternal plates of the 2.5 cm. sternum, of which GADOW speaks, seem to be the somewhat rounded medial edges of the sternal plates.

In an embryo about to hatch, with a sternum 4.2 cm. in length, the sternum is strongly arched with a long attenuated xiphisternum and a postero-lateral process extending as far caudad as the xiphisternum. The sternum proper is wholly devoid of special structures, just like the adult sternum at my disposal[2].

Highly characteristic of the development of the sternum in the ostrich is *the total absence of anything that could reasonably be considered to belong to a rudimentary sternal keel of the carinate type.* This conclusion is in full accord with that expressed by Miss LINDSAY (1885) and LOWE (1928).

The very small measure of agreement between the ossifications of the sternum in the Ratites and the Carinates has been emphasized especially by LOWE (1928), and I do not consider it necessary to indicate all the differences, but shall confine myself to mentioning that there are in the Ratites but one centre (or two) on each sternal plate. In the Carinates there are many centres, of which one belongs to the sternal keel. There is no homologon to this centre in the Ratites.

When examining the pterylosis of *Struthio,* one is struck by the configuration of the ventral apterium in embryos from 21 days and upward. In the 21-day *Struthio* embryo this apterium is frontally fairly narrow, then after widening suddenly below the sternum it becomes narrow again. This widened part of the apterium is where the *callositas* will appear later. The callositas has been described by LOWE (1926, 1928) as an oval portion of the skin which is thickened to form a thick sole outside the sternum.

When an ostrich rests, it sits with its tarsi flat on the ground and with the breast leaned against it. During the long sitting periods of the ostriches, during which the breast constantly rests on the ground, the sole apparently has an important function. LOWE holds the view that the callosity may be a reptilian heritage, which seems to me fairly probable owing to its early appearance in the

[1] This depressed portion of the metasternum thus lies at the bottom of a shallow pit. The pit probably corresponds to the pit between the protuberance which various writers have described from adult sterna.

[2] I cannot avoid the supicion that the way in which some at least of the sternal structures are illustrated by previous authors, is due to their material having dried in the process of preservation or during the investigation.

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embryo. As, then, the breast of the ostrich thus rests heavily on the ground and the sternum on the breast-sole, would it be too far-fetched to imagine that the sternum has answered in some way to the pressure, for instance, by its arched form or by occasionally developing the protuberances which have been interpreted as keel-rudiments?

(4) *Coracoid, procoracoid, scapula, clavicula, and episternum.*

LOWE (1928) has published a very interesting explanation of the importance attaching to the coraco-scapular bar for deciding the question of the flying or non-flying ancestors of the struthious birds, and I have very little to add to his evidence of the primary flightless state of these birds.

LOWE laid stress on the fact that the coraco-scapular bar of the Ratites agrees very closely with that of the Pseudosuchians and Coelurosaurs. He said (p. 223): "In this remarkable similarity, then, there seems to be justifiable ground for, at any rate, a preliminary assumption that the morphology of the coraco-scapular bar in the Struthiones is reptilian or primary, and not secondary to a loss of flight, as has been so often maintained." On the other hand, the development of the coraco-scapular bar in the Carinates was due to the development of strong flying muscles, and was thus a secondary acquisition. Generally the Carinates have a long coracoid, though there are some exceptions. The Ratites always have a short one.

The relation of the coracoid, procoracoid and scapula to one another in the Struthiones is still not quite correctly interpreted. Miss LINDSAY (1885) found that these parts are separate rudiments in a 4-day ostrich embryo. Here Miss LINDSAY must have mixed up the stages, because there is no trace of any skeletal rudiments present in a 4-day stage. Nevertheless, her observation seems to be fundamentally correct, as in a 9-day stage these parts are represented by blastemic or prochondrial condensations. Moreover, in a 10-day stage, at which the shoulder girdle is already chondrified, the three parts are separated from one another by very distinct lines of fusion. The procoracoid, however, forms a process corresponding to the procoracoid process of the scapula, as described by BROOM (1906). Later the lines of fusion disappear and the coraco-scapular bar appears as a single cartilage. There is thus strong doubt as to the one-ness of the coraco-scapular bar. It is premature to conclude from that evidence that there is any fundamental difference between the bar in the Ratites and the Pseudosuchians because after ossification the bar is unitary in both cases. From the type of coracoid present in *Archaeopteryx* LOWE considered that it clearly represents a pre-volant stage in avian evolution. He cannot then believe that the long and slender bones of the manus could possibly support the strain of the beating of 10 or 12 well-developed primaries.

As explained above, the development of the wings in the struthios birds as a whole must be considered a regressive one, beginning with *Struthio* and *Rhea* as

the most primitive and *Casuarius, Apteryx* and moas as the most secondarily transformed. This opinion opposes that of LOWE, according to which *Struthio* and *Rhea* (among the Ratites) have proceeded farthest towards a volant condition. Evidence substantiating my view is found in the fact that the Saururae and most Coelurosaurs had three (II, III and IV) free fingers and metacarpalia. Therefore it is obvious that the ancestors of the Carinates and Ratites must also have had these free fingers and metacarpalia. In *Struthio* those fingers and the distal end of the metacarpalia 3 and 4 are free. In addition, *Struthio* has a phalangeal formula which agrees with that of the Saururae except in regard to the 4th finger, which may perhaps have only two phalanges instead of the four present in the Saururae. It must, however, be remarked that the 4th finger in the Saururae is also (somewhat) reduced. In the Carinates, *Opisthocomus* has the same phalangeal formula as *Struthio* and 3 free fingers, but the 4th metacarpale is distally joined to the 3rd. In *Rhea* a certain progressive development has occurred, as the metacarpalia 3 and 4 are united distally and the 1st phalange of the 3rd finger is broadened. In the other struthious birds the wing is more or less degenerate. In the Carinates, too, there is, of course, a reduction, but this reduction also means an effective adaptation for flight.

Assuming that the Ratites were degenerate flying birds, with a more or less carinate coracoid and scapula, it would be very unlikely that the degeneration of the coraco-scapular bar could have resulted in a shoulder apparatus almost identical with that present in ancestral reptile groups. As LOWE (1928, p. 230) has pointed out, such a result would be "inconceivable, since it is not only seen in those avian types in which the power of flight has been obviously lost, but we also have to account for the presence of the foramen coracoideum and the precoracoidal element in the complex." If the coracoid apparatus is not of carinate origin, but a primitive reptilian structure, it is quite clear that the absence of acromion and acrocoracoid may also be primitive.

It is generally declared that the clavicle and episternum of the Pseudosuchians have disappeared in their successors, the Coelurosaurs. In the preceding pages I have suggested, however, that these skeletal parts were not lost, but were preserved, though in such a state that they could not appear in the fossils. They might be unossified membrane structures or perhaps chondrified, like the similarly absent sternum. Maybe they were not even preserved in the living specimen in that state but only as a transitory rudiment in the embryo. But that would be a sufficient reason for their possible reappearance or rejuvenation in the birds, after a long period of apparent absence in the Coelurosaurs. The conditions in *Struthio* embryos may to some extent serve to illustrate this idea. In the adult ostrich there is no trace of a clavicle or an episternum. But in the 13- and 15-day embryos both of them, or at least the clavicle, seem to be represented by distinct structures. In that stage of development the sternal plates

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are still widely distant caudally from each other, but frontally they meet in the medial line. Between the plates we find the linea alba. Frontally this thread of coarse connective-tissue fibres enters between the frontal ends of the sternal plates, but before that the thread has changed into a triangular, vertical fibre plate, the base of which is directed frontad, where it meets and connects on each side with a rudimentary clavicle. As the linea alba in reptiles runs frontad between the sternal halves and frontally connects with the episternum, it seems possible that the differentiated frontal part of the linea alba in the ostrich represents the episternum. The rudiments of the clavicles are fairly strong, dense, well-defined bundles of connective tissue, bordering the coracoid frontally and extending to the frontal end of the scapula. The reason why I consider these membranes to be clavicles is the occurrence in them of isles of very strongly stained tissue very closely reminiscent of chondrial or osteogenous matter. In *Dromaeus* a corresponding rudiment has apparently furnished this bird with its ossified rudimentary clavicle. In ostriches a few days older the rudimentary clavicles and the "episternum" have disappeared. Perhaps the conditions in the ostrich embryo can throw some light on those in the pro-ratite birds and the Coelurosaurs, the remains of which do not reveal any clavicles at all. My opinion is that the clavicles of those animals were either non-ossified rudiments in the adults or rudiments appearing only during the ontogenesis.

There is thus no evidence to show that the struthious birds have lost their clavicle through the degeneration of the carinate furcula.

(5) *The pygostyle.*

It would be quite natural if, when the tail became shortened and consequently the tail feathers became concentrated within a short portion of the caudal vertebrae, this portion should sometimes at least have been strengthened in some way, independently of a near relationship with other birds. It is conceivable that the metaptile plumes of the struthious birds had some use for a strong support in the same way as the teleoptiles of the flying birds — and that is, in fact, what has taken place with regard to the pygostyle. In pygostyles of *Struthio,* illustrated by MIVART (1874), and BEEBE (1907), the last vertebrae have fused particularly strongly with their spinal processes, whereas the rest of the vertebrae are comparitively free. It is the fused dorsal parts that give the strong support to the tail feathers. In the carinate birds the support of the rectrices, for instance in *Larus,* is provided by the strongly developed hypocentra of two vertebrae at the frontal end of the pygostyle. The hypertrophical development of these two centres causes the well-known dorsal bend of the pygostyle[1]. It also causes the phenomenon that the extreme end of the tail is located at the upper end of the pygostyle. *Thus the pygostyle of* Struthio *and that of carinate*

[1] In auks and grebes the pygostyle is not very highly developed, a fact that may be due to a secondary reduction.

birds are not homologous. This statement fully accords with the fact that the pygostyle is formed in the early embryo in the Carinates but in old adults in the ostrich.

(6) *Other reasons why the Ratitae are not degenerate Carinatae.*

It has been said that the less pronounced difference in strength between the ulna and the radius in the Ratites affords evidence of their descent from flying birds. In reply to this evidence it may be observed that in the compsognathid Coelurosaurs the ulna and the radius differed very little in strength and that the conditions in the Ratites could be easily explained as inherited from their ancestors, all the more so as the embryonic development of the forearm and the wrist of *Struthio* differs from that in the Carinates. As has already been demonstrated, the head of the ulna in the Carinates turns inwards, causing the ulnar abduction, whereas in *Struthio* no such inward turning takes place. The ulnar abduction of the hand of *Struthio* develops much later than in the Carinates and is not so definite, as for a long time in older embryos it is replaced by a radial abduction (!).

The pneumaticity of the bones is said to have become partly lost in the Ratites as they degenerated from the Carinates and became cursorial birds. The fact that the pneumaticity had already occurred in the Coelurosaurs is enough to throw some doubt upon that conclusion: pneumatic bones probably occurred in the ancestors of birds.

Regarding the pterylosis, it is not possible to decide which type is the primitive, which the secondary changed one. As there are similar principal apteria in the ostrich embryo as well as in that of the Carinates, the conclusion is perhaps permitted that these apteria were also present in their ancestors. This question, however, cannot be answered in a satisfactory way.

The alleged degeneration of the feather covering of the struthious birds has already been considered in preceding chapters.

The authors who derive the Ratites from the Carinates consider that the oil gland disappeared in the former except, however, in *Apteryx* — in connection with the degeneration of the carinate feather covering. I cannot express any opinion about the existence of the oil gland but would only suggest that the very different mode of life led by the larger species on the one hand and the small *Apteryx* on the other, may be sufficient to explain the loss of the oil gland in most Ratites and its preservation in *Apteryx*. Considering this possibility, I must express it as my personal opinion that the ancestors of the birds had an oil gland, which disappeared in most Ratites but was preserved in the Carinates and in the *Apteryx* line of development.

Concerning the palatal region Lowe (1928) stated that the conditions seen in the adult Ratites generally occur in embryonic stages of the Carinates and, when the ratite conditions are found in Carinates, the embryonic development

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must have stopped at the struthious stage. LOWE also pointed out that the large pterygoid process is a primitive character of the Ratites as well as of some carinate groups. Their absence in most Carinates is a secondary feature.

Explanation of letterings

c 1—5 centralia 1—5
ca 1—5 carpalia 1—5
c.bl central blastema
F fibula
f fibulare
i intermedium
p.asc processus ascendens
Ph prehallux
pis pisiforme
Pp prepollex
R radius
r radiale
r.bl radial blastema
T tibia
t tibiale
U ulna
u ulnare
I—V digits 1—5

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ON THE ORIGINAL MAMMALIAN CARPAL PATTERN

BY

NILS HOLMGREN†

In my paper "An embryological analysis of the mammalian carpus and its bearing upon the question of the origin of the tetrapod limb" (1952) I treated a large material in order to arrive at an idea of the original mammalian carpal pattern.

The result of this analysis was an interpretation of the mammalian carpus that did not agree fully with the generally accepted one. The problems concerning which agreement could not be established were (1) the question of the intermedium and lunatum, and (2) the question of the ulnare and centrale 4.

(1) The first of these questions was answered by the statement that there are in many mammals a separate or nearly separate intermedium present together with a centrale 1, the lunatum being the intermedium + centrale 1 (centrale proximale). Such mammals are *Pedetes* and *Bathyergus, Tupaia, Elephantulus, Talpa, Sorex, Dasypus, Viverricula, Galago, Nycticebus, Tarsius* and possibly *Pipistrellus, Centetes, Manis, Procavia, Tragulus, Microcebus,* and *Macacus.*

(2) The second question was answered by KINDAHL who interpreted the *processus styloideus ulnare* (ulnar process, head of the ulna) as the real ulnare and the element called the ulnare by the present author as the centrale 4. KINDAHL found the process as a separate or nearly separate rudiment in *Erinaceus, Centetes, Ericulus,* and *Talpa.* To these statements may be added that I have found a separate process in *Didelphis,* a nearly separate one in *Echidna,* and at least a somewhat delimited process in a large number of other mammalian embryos, such as those of *Funambulus, Pedetes, Galeopithecus, Manis, Felis Phoca, Sus, Bos, Tragulus* and *Microcebus.* Thus it must be concluded that the ulnar process is the real ulnare homologous with the ulnare of reptiles and that the element called the ulnare by the present author is the centrale 4, likewise present in the reptiles.

The original carpal pattern of the mammals is, generally, the same as that of the recent reptiles, though certain reductions are common in the latter, especially concerning the radiale. The ulnare is generally well developed. In the crocodiles it forms a strong column.

When to make a comparison with the mammal-like reptiles of the Permian and Triassic I have chosen a Gorgonopsid, *Lycaenops* from the Upper Permian, a Cynognathid, *Leavachia,* and a Therocephalian, *Theriodesmus,* as suitable representatives of those forms.

These three genera seem to supplement each other in a satisfactory way.

In *Lacvachia,* described by A. S. BRINK and J. W. KITCHING, the mammalian pattern is almost diagrammatically developed. The ulnare is a separate element. The intermedium and proximal centrale are separate elements. The centralia 2 and 3 are fused. The centrale 4 is a columnar body. The only doubt about the interpretation of this carpus concerns the ulnare, which possibly could represent a dislocated pisiforme. However, this is not the case as I was able to state when investigating in 1952 the type material in the BERNARD PRICE collection in Johannesburg, South Africa. The keeper of these important collections, Dr. A. S. BRINK, at my demand kindly made an additional dissection below the ulnare. It was stated thereby that a pisiforme process was present below the level of the ulnare. If there, however, should remain any doubt about this identification I need only to point out that in SEELEY's description of the hand of *Theriodesmus* there is a quite normal ulnare with a pisiforme present that is in contact with the ulna. In addition, there are three distal centralia present, but the intermedium and the proximal centrale are fused with the radiale to form a scapho-lunatum (SEELEY). The carpus of *Lycaenops* as described by BROOM is almost identical with that of *Leavachia* though the ulnare is missing but a pisiforme present.

In these three carpi of mammal-like reptiles all the elements are present which constitute the mammalian carpal pattern according to the testimony of the embryological investigations.

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